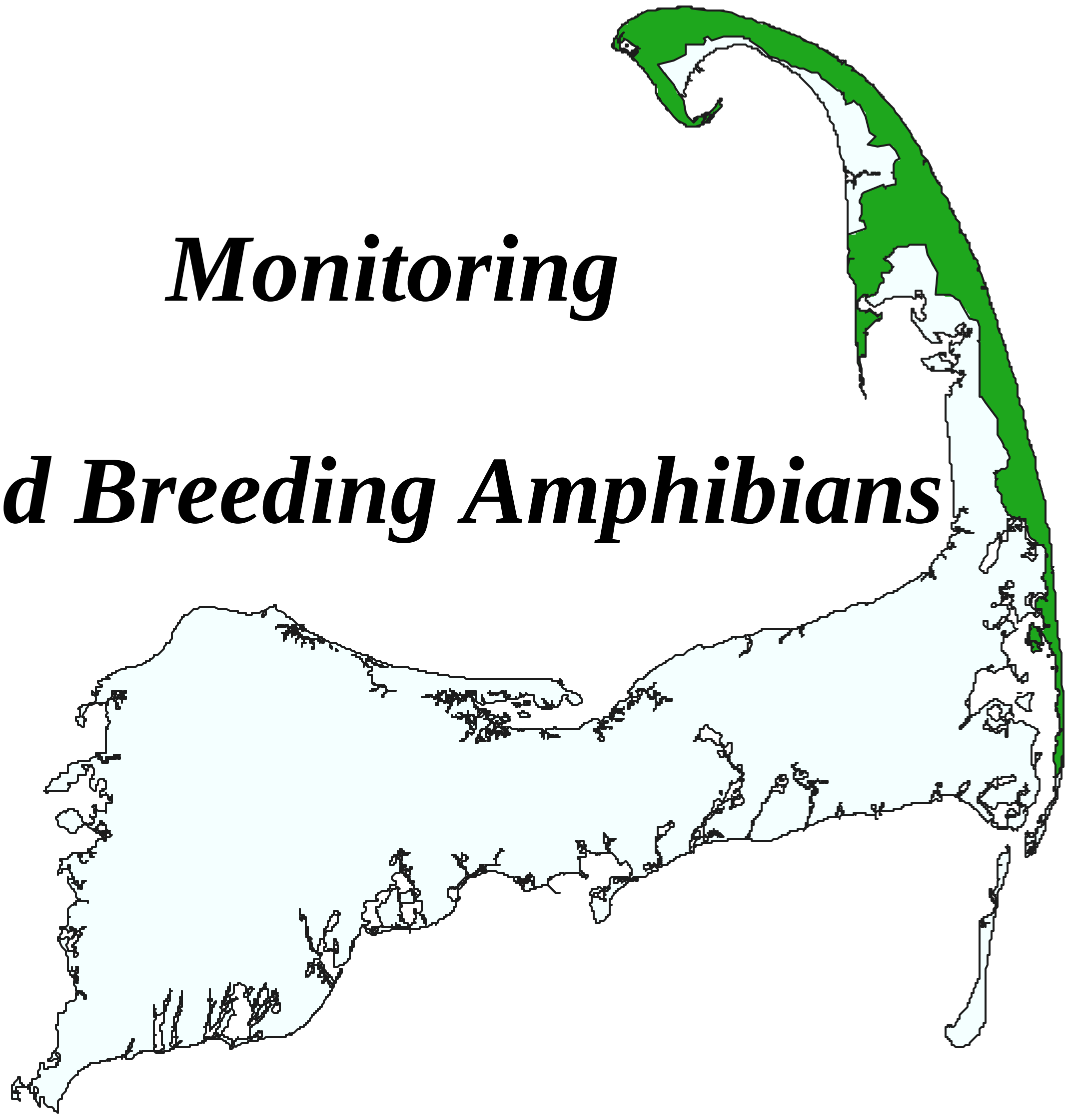
Monitoring

Pond Breeding Amphibians



USGS Patuxent Wildlife Research Center

Cape Cod National Seashore

MONITORING POND-BREEDING AMPHIBIANS

A Protocol for the Long-term Coastal Ecosystem Monitoring Program
at Cape Cod National Seashore

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Long-term Coastal Ecosystem Monitoring Program
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PREFACE

Overview of the Long-term Monitoring Program

Cape Cod National Seashore serves as a National Park Service prototype monitoring park for the Atlantic and Gulf Coast biogeographic region. The USGS, in cooperation with the National Park Service, is charged with designing and testing monitoring protocols for implementation at Cape Cod National Seashore. It is expected that many of the protocols will have direct application at other Seashore parks, as well as U.S. Fish and Wildlife Service coastal refuges, within the biogeographic region.

The Long-term Coastal Monitoring Program at Cape Cod National Seashore is composed of numerous protocols that are relevant to the major ecosystems types (Estuaries and Salt Marshes, Barrier Islands/Spits/Dunes, Pond and Freshwater Wetlands, Coastal Uplands). The pond-breeding amphibian protocol is associated with the Pond and Freshwater Wetlands component of the monitoring program. The overall program is designed so that all of the protocols are interrelated. For example, information acquired from the water quality protocol or fish distribution protocol may be especially relevant to interpreting observed trends for pond-breeding amphibians. Roman and Barrett (1999) present a conceptual description of the entire monitoring program.

Protocol Organization

To maintain some consistency among the various monitoring protocols, each protocol is organized as follows. PART ONE of the protocol is intended to provide detail on the objectives of the monitoring protocol and to provide justification for the recommended sampling program. Extensive incorporation of relevant literature and presentation of data collected during the protocol development phase of the project are used to justify a particular sampling design, sampling method, or data analysis technique. PART TWO is a step-by-step description of the field, laboratory, data analysis, and data management aspects of the protocol.

Executive Summary

During the 2001 field season, we assessed a variety of field techniques that could be used to quantify long-term population trends of amphibians at Cape Cod National Seashore. We used manual anuran call surveys, conducted from March to September, to assess seasonal variation in anuran call rates and to assess occupancy rates at different wetlands on the Cape. We used automated data recorders to assess diel variation in anuran call rates. We used egg mass counts as an index to population size to monitor wood frog *Rana sylvatica* and spotted salamanders *Ambystoma maculatum*. We attempted to dye tadpoles of green frogs *R. clamitans* to assess using mark-recapture models to quantify larval populations sizes at breeding ponds. We conducted nocturnal road surveys to quantify the spatial distribution and relative abundance of rarer species, including eastern spadefoot (*Scaphiopus holbrookii*), Fowler's toad (*Bufo fowleri*) and four-toed salamander (*Hemidactylium scutatum*). This was one of the first studies that simultaneously quantified temporal and environmental variation in anuran advertisement calls, knowledge that is essential when designing a statistically reliable monitoring program. We used an information-theoretic approach to assess seasonal variation in detection probabilities (p) and wetland occupancy rates (Ψ) and circular statistics to quantify diel variation in calling behavior.

Three survey windows were needed to monitor all species with call surveys. Detection probabilities within survey windows ranged from 0.47 for pickerel frogs (*Rana palustris*) to 0.81 for spring peepers (*P. crucifer*) and green frogs (*R. clamitans*), and wetland occupancy rates ranged from 8% for wood frogs (*R. sylvatica*) to 93% for spring peepers. All species had peak detection periods <4 hrs after sunset, which agrees with current North American Amphibian Monitoring Protocol (NAAMP) guidelines. However, we were unable to detect one species, eastern spadefoot (*Scaphiopus holbrookii*), therefore call surveys may not be appropriate for all species. Calling behavior of anurans was more sensitive to surface water temperature than air temperatures, which suggests that surface water temperature constraints may be necessary. These results show that quantifying temporal and environmental variation in anuran calling behavior at a local scale are critical before embarking on a monitoring program using call surveys.

Egg mass counts could be used to monitor wood frog and spotted salamander populations in the park, as they take relatively little time to conduct and provide precise indices. Using automated recording systems (ARSs) is not a practical technique to incorporate into a long-term monitoring program, although data gathered from ARSs was extremely important in determining survey windows. We were unsuccessful using tadpole dyeing to assess larval population sizes because we had difficulties with excessive mortalities and were often unable to detect marked animals when dye concentrations were low. Dip netting was a relatively time-consuming technique that required skilled observers to identify larvae, thus it is not practical as a long-term monitoring technique. Nocturnal road surveys were the only effective method we used to detect eastern spadefoot toads, particularly in the Provincetown area. Because this is a state-listed species, more research is needed to determine how road surveys could be designed to monitor spadefoot toads.

Based on our fieldwork, we recommend that the Park Service utilize anuran call surveys and egg mass counts to monitor pond-breeding amphibians within Cape Cod National Seashore. These are cost-effective techniques and will provide statistically reliable estimates of population trends. In addition, we recommend that further research into the feasibility of using nocturnal road surveys to monitor trends of eastern spadefoot toads be conducted.

TABLE OF CONTENTS

Table of Contents continued

Table of Contents continued

APPENDICES

LIST OF FIGURES

List of Figures continued

LIST OF TABLES

ACKNOWLEDGEMENTS

Development of this protocol was supported by the USGS-Biological Resources Division, with funds administered by the USGS Patuxent Wildlife Research Center at the University of Rhode Island. Special thanks are extended to the Superintendent and natural resource management staff at Cape Cod National Seashore (CACO) for providing logistical support. In particular, we want to thank Robert Cook at CACO for assisting us with the initial study design and showing us important amphibian breeding habitat at Cape Cod, and Nancy Finley for assisting us with the NPS bureaucracy. Robin Jung, USGS Patuxent Wildlife Research Center, graciously lent us automated recording systems to monitor frog calls and let us use her dyeing protocols to quantify tadpole population sizes. We thank Mary-Jane James-Pirri for proof editing this report. Cover photographs are courtesy of Scott Egan (eastern spadefoot toad and spotted salamander) and Charles Roman (freshwater pond).

PART ONE
Background and Justification for the Amphibian Monitoring Protocol

INTRODUCTION

Amphibians can be a useful indicator of environmental change because they are sensitive to habitat perturbations and other human-induced change (Blaustein and Wake 1990). Reports of amphibian declines throughout the world are currently a major concern for biologists (Pechmann *et al.* 1991). Reasons for these declines have been theorized to include habitat fragmentation (Dickman 1987; Laan and Verboom 1990), increased UVb radiation (Hays *et al.* 1996), episodic acidification (Vertucci and Corn 1996), exotic disease (Laurence *et al.* 1996), road mortalities (Jackson and Tyning 1989; Fahrig *et al.* 1995), and increased predation pressure (Drost and Fellers 1996). However, much of the evidence reporting amphibian declines is anecdotal, as few long-term amphibian monitoring programs have been initiated.

Acid Rain

Due to glacial processes, the Lower Cape is mainly comprised of sandy soil and dominated by pitch pine (*Pinus rigida*) forest. Due to the high permeability of sandy soil, water and nutrients leach fairly easily through the soil. This in combination with the fact that coniferous litter (in this case pitch pine litter) releases acidic compounds, causes the ponds on the Seashore to retain a highly acidic pH. While many of these ponds sampled were above the current pH threshold for many species present on the Cape, an increase in acid rain could have a significant impact on future populations. For example, Portnoy (1990) found that spotted salamander (*Ambystoma maculatum)* populations on the Seashore were adapted to acidic conditions, but embryos developing in ponds that were very acidic (4.3-4.5), and had high tannin-lignin concentrations were subject to embryonic mortality and embryonic malformations. Additionally, Fowler's Toad (*Bufo fowleri)* embryonic mortality occurs when breeding pond pH is less than 4.1 (Freda and Morin 1984). Therefore, acid rain, although not currently an apparent problem on the Cape, can potentially lead to amphibian declines.

Amphibian use of seasonally-flooded ponds

There are 11 species of amphibians that have been recorded in the Seashore (Tyning *et al.* 2000 unpubl. data; Table 1), of which 8 species breed primarily in ephemeral, seasonally-flooded, or semi-permanent ponds. Tadpoles of two anurans, green frog *Rana clamitans* and American bullfrog *R. catesbeiana,* take 1-2 years to undergo metamorphosis in southern New England, therefore they require ponds with relatively permanent hydroperiods for breeding (Paton and Crouch 2002). A third species, eastern red-backed salamander (*Plethodon cinereus*) is a widespread terrestrial breeder and has completely different breeding habitat requirements than the other species found in the Seashore (Klemens 1993). At least three species found on the Seashore are obligate breeding species in free-fish ponds, wood frogs (*Rana sylvatica*), Eastern spadefoot toad (*Scaphiopus holbrooki*), and spotted salamander (*Ambystoma maculatum*); the other pond breeding species can be found in ponds with varying hydroperiod lengths (Klemens 1993; Snodgrass *et al.* 2000*a*, 2000*b*; Egan 2001, Paton and Crouch 2002).

Increased housing and groundwater wells on the Cape could affect amphibian breeding pond hydroperiod, which could negatively impact amphibian community structure at the Seashore. Anthropogenic alteration of the hydroperiod at breeding ponds has reduced recruitment in *Bufo fowleri* populations at Trustom Pond National Wildlife Refuge and at the Goosewing Beach Nature Preserve in Rhode Island (Tupper, unpubl. data). Because declines in *B. fowleri* have been documented in other areas as well (Sanders 1970, Lazell 1972, Green 1989, Cook pers. comm.) and since *B. fowleri* is relatively common on the Cape, long term monitoring of anurans such as *B. fowleri* may be of particular importance in assessing environmental health on Cape Cod NS.

Table 1. Occurrence of anurans in four towns on Cape Cod National Seashore based on previous collections and surveys by Rich in 1960 (unpubl. data), Lazell (1972) (*), Tyning *et al.* (2000 unpubl. data) (X); and anecdotal information (A).

Species*	**Eastham**	**Wellfleet**	**Truro**	**Provincetown**
Spotted Salamander (*Ambystoma maculatum*)	X	X*	X	
Red-spotted Newt (*Notophthalmus viridescens*)	X			
Eastern red-backed Salamander (*Plethodon cinereus*)	X*	X*	X*	X*
Four-toed Salamander (*Hemidactylium scutatum*)		X	X	
Eastern Spadefoot Toad (*Scaphiopus holbrooki*)	X*	X	X	X
Fowler's Toad (*Bufo fowleri*)	X*	X*	X*	X*
Spring Peeper (*Pseudacris crucifer*)	X*	X*	X*	X*
American Bullfrog (*Rana catesbeiana*)	X	X*		
Green Frog (*Rana clamitans*)	X	X*	X*	X*
Wood Frog (*Rana sylvatica*)	X	A		
Pickerel Frog (*Rana palustris*)	A	A*	X*	A

Nomenclature follows Crother 2001.

Certain species of amphibians require seasonally-flooded ponds as breeding habitat (*e.g.*, *Rana sylvatica*), where adults mate and oviposit, and larvae develop until they undergo metamorphosis (Paton and Crouch 2002). For those amphibian species that usually breed in seasonally-flooded ponds, ponds with a permanent hydroperiod are usually unsuitable habitat because fish may be present that prey on their larvae, and other species of amphibians that favor permanent hydroperiods could be present that are potential competitors (i.e., *Rana clamitans*, *Rana catesbeiana*) (Kenney and Burne 2000). The duration of the larval period to metamorphosis is highly variable among species and can fluctuate within a species as a function of different densities of competitors and predators (Wilbur 1972, 1980; Paton and Crouch 2002). For example, Wilbur (1972) found the larval period for spotted salamanders ranged from 91 to 122 days in the presence of different densities of potential competitors. The timing of pond drying can also affect the length of the larval period of some amphibian species (Wilbur 1987; Semlitsch 1987; Semlitsch and Wilbur 1988; Skelly 1996), and is critical for the successful recruitment of explosive breeders such as *Scaphiopus holbrookii*.

Amphibian species richness and productivity in seasonally-flooded breeding ponds (i.e., ponds that have surface water for 2-9 months annually) are positively correlated with hydroperiod (i.e., the number of days a pond has surface water annually) in experimental settings (Wilbur 1987; Rowe and Dunson 1995) and in natural populations (Pechmann *et al.* 1989; Semlitsch *et al.* 1996; Snodgrass *et al.* 2000*b*; Egan 2001, Paton and Crouch 2002). The importance of hydroperiod in a natural population was shown by Semlitsch *et al.* (1996), who monitored a pond in South Carolina over 16 years that was inundated an average of 170 days annually (range 3 to 391 days). They found that years with short hydroperiods (≤100 days) resulted in total reproductive failure, while years with long hydroperiods (>200 days) tended to have the greatest diversity and productivity. Because pond-breeding amphibians are dependent upon the timing of the hydroperiod, any significant change to the hydroperiod of ponds on Cape Cod NS could significantly affect larval metamorphosis and annual recruitment. The hydroperiod at amphibian breeding ponds could be a critical issue at the Seashore, where there is the potential for deep wells to be used as water sources for towns or individual home owners which could possibly affect ponds used by breeding amphibians. This question needs further investigation.

In addition to the potential desiccation of amphibian breeding habitat via consumption of groundwater by homeowners, the timing of inundation may also be affected. Inundation of temporary ponds is an important characteristic of amphibian breeding sites that has received less attention from biologists and regulators. It is critical that ponds are flooded at the appropriate time of year to meet the life history requirements for amphibian species that could potentially breed at the site (Semlitsch 1985; Pechmann *et al.* 1989; Paton and Crouch 2002). Because many amphibian species often breed at the same pond, amphibians use temporal segregation to avoid competition and predation pressure in breeding ponds (Blair 1961; Wilbur 1980, 1987). Yet, few studies have quantified the movement phenology of natural populations of pond-breeding amphibians, including information on both the timing of immigration by adults and emergence by metamorphs (but see Murphy 1963; Paton and Crouch 2002, Crouch and Paton 2002) . Usually only qualitative information on seasonal variation of movements is available for most parts of North America, particularly for adult anurans (Wright and Wright 1949; Klemens 1993; Semlitsch *et al.* 1996). Hence, quantitative, statistically relevant data collection and analysis will produce valuable results and will more accurately describe amphibian relationships with environmental factors at Cape Cod NS.

Chemical Threats

The potential for runoff effects of fertilizer or herbicides/pesticides from residences or commercial developments in the vicinity of amphibian breeding ponds is apparent on the Cape. *Bacillus thuringiensis*-based pesticides (which are used on the Cape) could negatively impact *Bacillus*-sensitive amphibian populations in the region, although available evidence suggests that these pesticides would have minimal impact on amphibians (Buckerner *et al.* 1974; Bellocq *et al.* 1992; Charbonneau *et al.* 1994; McClintock and Schaffer 1995). However, Lazell (1972) attributed Fowler's toad extinctions on Nantucket to insecticides, and Breden (1988), Cook (pers. comm.), and Sanders (1970) have attributed anuran declines to chemical pesticide applications. The effects of non-*Bacillus* based pesticides and mosquito larvacide applications on amphibian populations on Cape Cod are unclear, and exemplify a need for long-term amphibian monitoring at the Cape Cod NS.

Potential for Road Mortality

Vehicles have major impacts on amphibian populations as well, particularly on rainy nights near amphibian breeding ponds (Van Gelder 1973). Roads are among the most important landscape features thought to affect amphibian dispersal among patches (Mader 1984; Fahrig *et al.* 1995; Forman and Alexander 1998), and overall species diversity (Findlay and Houlahan 1997; Lehtinen *et al.* 1999). Forman and Alexander (1998) estimated that roads might ecologically impact up to 15-20% of the United States. Previous research has shown that increased road density near a *Rana arvalis* breeding pond was severely detrimental to this species (Vos and Chardon 1998). Heavy traffic at the Cape during the tourist season in the summer months could result in high anuran road mortality of adults (*Rana clamitans, Rana catesbeiana*, *Scaphiopus holbrookii*, *Bufo fowleri*) or metamorphs emigrating from ponds (*Rana sylvatica, Ambystoma maculatum, Scaphiopus holbrooki, Bufo fowleri*).

Eutrophication, Wildfire, and Fishing

Increases in the number of humans living on the Cape could also result in nutrient enrichment (eutrophication) of ponds and wetlands. In the Pacific Northwest, certain species of amphibian larvae experience high mortality rates when exposed to nitrate levels at 5 mg $N\text{-}NO_2^-$/L (the U.S. Environmental Protection Agency recommended limits for warm-water fishes) (Marco *et al.* 1999). With increasing suburbanization of the Cape, there is potential for eutrophication of ponds, via chemical runoff from adjacent lawns or leaking septic systems.

With a reduction in fires on the Cape, extensive stands of pine are becoming dominated by oak. The impacts of this large-scale conversion of vegetation on amphibian populations on Cape Cod are unknown. Freda and Morin (1984) hypothesized that a reduction in wildfire for subclimax community habitat specialists (i.e., *Hyla andersoni*) that breed in and inhabit low pH ephemeral seeps, bogs, and temporary ponds in pitch pine barren forests in southern New Jersey will result in an elimination of a shrub or herbaceous zone of breeding ponds, and will be a confining regulatory factor in species' distribution. Therefore, long term monitoring and management techniques are essential for the protection of these habitat specialists on protected land. Since the Cape Cod and southern New Jersey pitch pine forests share habitat similarities, it is possible that the conversion of pine forests to oak-dominated forests may limit or restrict species' distribution in areas that were once maintained by periodic wildfire at Cape Cod NS.

Many ponds at Cape Cod NS have been stocked, either intentionally or unintentionally, with non-native fish, and these introduced predators can have a detrimental impact on amphibian populations as demonstrated by Drost and Fellers (1996).

Prior Monitoring Efforts

In 1991, the World Conservation Union established the Species Survival Commission, which formed the Declining Amphibian Populations Task Force (DAPTF). The DAPTF operates worldwide to gather information on amphibian population declines and determine their possible causes. In 1994, as part of this worldwide effort, the North American Amphibian Monitoring Program (NAAMP) was established by the Biological Resources Division of the U.S. Geological Service (NAAMP 2002). The primary goal of NAAMP is to help develop a standardized monitoring program in the United States and Canada that assesses changes in the spatial distribution and relative abundance of amphibians at state, provincial, regional, and continental scales.

Surprisingly, little is known about the long-term population dynamics of pond-breeding amphibian populations in North America. We know of only one published quantitative study (Pechmann *et al.* 1991) that has monitored populations of pond-breeding amphibians for over 15 years. Therefore, few quantitative data exist that have investigated the effects of factors such as urban sprawl, pollution, roads, and habitat fragmentation (Semlitsch and Bodie 1998) on the population dynamics of amphibians in North America. Thus, it is critical that long-term monitoring be used to understand how the aforementioned factors affect bioindicator species populations status on Department of Interior Parks and Refuges.

MONITORING QUESTIONS

The protocols developed from this proposal are designed to implement a monitoring program that will track long-term population trends of pond-breeding amphibians at Cape Cod NS. Although a number of techniques are available that could be used to monitor amphibian population trends at Cape Cod NS (*e.g.*, Berrill *et al.* 1992, Heyer *et al.* 1994, Corn *et al.* 2000), not all methods will work at Cape Cod. There is increasing interest in monitoring long-term population trends of anurans by assessing occupancy rates at breeding ponds with call surveys. One of the critical factors to consider when designing a monitoring program is ensuring that surveys take place when detection probabilities are high (Shirose *et al.* 1997, Elizinga *et al.* 2001). Therefore, developing a statistically reliable monitoring program is a challenge because adults of most anurans reside at breeding ponds briefly each year (Paton and Crouch 2002). Males display seasonal variation (Corn *et al.* 2000, Crouch and Paton 2002) and diel variation (Mohr and Dorcas

1999, Bridges and Dorcas 2000) in advertisement calling behavior. In addition, calling behavior can be affected by temperature (Mossman *et al.* 1998).

Widespread use of call surveys to quantify population trends of anurans was initiated by the North American Amphibian Monitoring Program (NAAMP), which uses volunteers to monitor randomly selected routes throughout North America (NAAMP 2002). Current protocols developed by NAAMP propose that observers initiate surveys at least 30 minutes after sunset and complete surveys by 0100 hr. However, Bridges and Dorcas (2000), working in South Carolina, found that at least one species, southern leopard frog (*Rana sphenocephala*), called consistently only from midnight until dawn, and detection probabilities of American bullfrogs (*Rana catesbeiana*) were greatest from midnight to 0700 hr. Thus, current NAAMP guidelines may not be appropriate for all species at Cape Cod. The problem is that little quantitative information is available on seasonal and diel variation in anuran calling behavior that could be used to develop monitoring protocols for anurans at Cape Cod (but see Crouch and Paton 2002). In addition, because there is tremendous latitudinal variation in amphibian activity patterns (Crouch and Paton 2002, Paton and Crouch 2002) we needed to assess seasonal and diel variation in male anuran advertisement calls to develop a monitor program that would be effective and practical at Cape Cod.

This monitoring program, if implemented, would provide information on population trends for most species of frogs in the park, although not all (*e.g.*, eastern spadefoot, see below). Biologists tracking anuran populations in the park with these protocols could look at interspecific differences in population trends. Because there are interspecific differences in hydroperiod requirement of each species (Paton and Crouch 2002), biologists could ask which anuran guilds are increasing or declining over time in the Park. If biologists tracked species occurrence at individual ponds over time, and habitats were also tracked over time, biologists could begin to address questions related to the effects of changes in habitat composition on anuran community structure on the Cape. Are there certain genera or species whose populations are more susceptible to habitat perturbation than others; and if so, what are these factors? What habitat characteristics seem to be most attractive and productive for particular species of amphibians? How dynamic are populations between years; and may this be leading us to false conclusions about possible declines in short-term studies? If vehicular traffic volume on the Cape were tracked, those data could be integrated with anuran population trend data to assess the impact of changes in traffic volume on amphibian diversity in the Park.

More specific types of research questions could only be addressed if research projects were initiated in the Park including: (1) What environmental changes are taking place that may be affecting amphibian populations?, (2) Are issues such as increased UVb radiation, habitat fragmentation, pond acidification, exotic disease, road mortalities, and increased predation pressures having an effect on population numbers, breeding phenology, and overall health of amphibians?, (3) What can we do to minimize the effect of increased anthropogenic change to the environment on amphibians?, (4) What is the direct impact due to road mortalities in amphibians? (this question is particularly important at Cape Cod given the high road mortality of eastern spadefoots we documented in the Provincetown area), (5) How do factors such as natural fires or fire suppression play a role in amphibian communities? (6) Is forest succession or lack thereof having an affect on the species composition of amphibian communities? (7) What can declines or increases in amphibian populations tell us about the health of our environment? (8) What effects are changes in hydroperiod of wetlands having on the community structure of amphibians?

SAMPLING METHODS

Project Objectives

The primary goal of this protocol is to assess the long-term population dynamics of pond-breeding amphibians at the Seashore. These data could be used to assess fluctuations in amphibian population status, changes in calling and movement chronology/phenology, and overall species richness in particular areas. It is our intention that these amphibian monitoring data could be dovetailed with data from other ongoing monitoring programs (biological and environmental), which would allow future researchers to begin to tease apart potential mechanisms driving population declines or increases if they are detected among amphibian populations. While developing this monitoring protocol, several amphibian monitoring techniques were tested throughout the Seashore. During protocol development, the following methodologies were tested:

(1) evaluating the effectiveness of calling anuran surveys to quantify adult anuran community structure at ponds at Cape Cod NS,
(2) quantifying the seasonal and diel phenology of anuran calling at Cape Cod NS,
(3) assessing the utility of using egg mass counts to monitor wood frog (*Rana sylvatica)* and spotted salamander (*Ambystoma maculatum)* populations,
(4) evaluating the effectiveness and practicality of using mark-recapture models to census anuran tadpole populations, when tadpoles are marked with neutral red dye,
(5) evaluating dip net sampling to quantify larval amphibian community structure at breeding ponds,
(6) evaluating the effectiveness of using minnow traps (0.63cm mesh) to sample red-spotted newt (*Notophthalmus viridescens)* populations, as well as the tadpoles of anurans associated with permanent hydroperiods (*e.g.*, American bullfrog (*Rana catesbeiana)* and green frog (*R. clamitans*)), and
(7) evaluating and optimizing a statistically viable data-collection network, eventually developing a system of long-term amphibian monitoring sites.

Manual Anuran Call Surveys

Site Selection

To assess the feasibility of using calling surveys to monitor changes in the spatial distribution of adult anurans at Cape Cod NS, we used a variation of the North American Amphibian Monitoring Program's (NAAMP) anuran calling survey protocol (NAAMP 2002). We established three calling survey routes on the Seashore from Eastham north to Provincetown. Ponds within each route were selected for monitoring based on the following criteria: (1) prioritizing ponds that were part of the existing hydrological monitoring protocol (Martin *et al.* 1993), (2) sampling ponds with a variety of hydroperiods, (3) sampling ponds from a variety of wetland types (see below), and (4) accessibility.

We first mapped the spatial distribution of all potential anuran breeding ponds based on 7.5 minute topographic maps for the Seashore (Burne 2001) and discarded any ponds that were not easily accessible via a footpath, bike path, or road. We then stratified ponds into two hydrological categories, (a) seasonally-flooded ponds that appeared to have no surface water for at least part of the year, or (b) permanent ponds with surface water throughout the year. Hydrologic categories were further subdivided into a series of wetland habitat types, *deep kettle ponds*--large, deep kettle ponds, with permanent hydroperiods, *shallow kettle ponds*--small, shallow ponds adjoining deep kettle ponds, generally with a permanent hydrology, *vernal ponds*--classic spring flooded ponds, that were seasonally-flooded, *dune slack ponds*--seasonally-flooded ponds dominated by cranberries and generally found in dune habitats, only found in the Provincetown area, *inter-dunal ponds*--which were large shallow, permanent ponds in Provincetown, *riparian marshes*--freshwater marshes and meadows associated with the Herring and Pamet Rivers, and *forested swamps*--red maple (*Acer rubrum*) and Atlantic white cedar (*Chamaecyparis thyoides*) forested swamps. Within each wetland habitat type, if there more than one example in a specific region of the park, we randomly selected a representative example of each wetland type for future sampling.

We established three calling survey routes, with 9-12 stops each as follows (see Appendix I and Portnoy *et al.* 2001 for characteristics and specific locations of each wetland; 31 total stations): **Route One** in Eastham and Wellfleet with 12 stops (*pond type is given in parentheses*): Grassy Pond (*shallow kettle*), W20 (*deep kettle*), W7 (*vernal*), Kinnicum (*deep kettle*), W18 (*vernal*), W17 (*cedar swamp*), Motel Bog (*cranberry bog*), E9 (*vernal pond*), E4 (*vernal pond*), E16 (*vernal pond*), E18 (*vernal pond*), and E15, Eastham (*red maple swamp*). **Route Two** (10 stops) in Wellfleet and Truro: W6 (*vernal pond*), Gull Pond (*deep kettle*), Herring Pond (*deep kettle*) Black Pond (*riparian marsh*) T14 (*vernal pond*), T15 (*vernal pond*), Snow Pond (*deep kettle*), Upper Pamet (*river*), Pamet Bog (*bog*), Ballston Marsh (*riparian marsh*). **Route Three** (10 stops) in Provincetown: Great Pond (*inter-dunal pond*), Lily Pond South (*inter-dunal pond*), Lily Pond Main (*inter-dunal pond*), P15 (*vernal pond*), P16 (*vernal pond*), P4 (*vernal pond*), P5 (*dune slack pond*), P8 (*dune slack pond*), P13 (*dune slack pond*), and P6 (*dune slack pond*). A fourth calling survey route was established and conducted to monitor green frog and bullfrog populations. Sites were chosen based on bullfrog and green frog presence. The ponds surveyed on **Route Four** were: E18 (Eastham), E9 (Eastham, *vernal pond*), E16 (Eastham, *vernal pond*), Kinnicum Pond (Wellfleet, *deep kettle*), Pamet Bog (Truro, *bog*), and the Upper Pamet River (Truro, *river*).

Sampling Frequency and Sampling Unit of Calling Surveys

We designed survey routes to sample at least three of each wetland habitat type during calling surveys. We surveyed only the nearest wetland to each fixed survey station. Survey routes took 1 observer approximately 3-4 hours to complete. Manual call surveys were conducted from 19 March through 15 September. Most stations were visited weekly from late March through mid-July. In addition, 12 stations in the southern part of the study area were surveyed weekly from 19 to 31 March to monitor wood frogs because the species has a restricted range on Cape Cod. From mid-July to 15 September, we surveyed 6 permanent ponds weekly to determine when green frogs and American bullfrogs ceased calling. Manual call surveys during March took place from 1200 to 1600 hr, when air temperatures were >10°C (50°F) when wood frogs were more likely to call (Crouch and Paton 2000, 2002). Surveys conducted from 1 April to 15 September took place from 30 min after sunset to approximately midnight and were conducted regardless of weather conditions. We varied the order that stations were visited between surveys to eliminate potential time biases. Each route was surveyed approximately once every seven days from 19 March through 11 July. Route Four (a route designed specifically to monitor green frogs and bullfrogs) was surveyed weekly from 18 July to 5 September to quantify seasonal variation in calling for these two species that breed in permanent ponds. On occasion, we had two observers survey separate halves of the route simultaneously.

Data Management

At each survey station, we monitored calling activity for 5 min (Current NAAMP protocol guidelines, L. Weir, NAAMP program coordinator, Patuxent, MD, pers. comm.). Observers recorded the maximum number of males calling and the maximum calling index at 60-second intervals, which resulted in a total of 5 data points collected at each stop. We used a categorical index to quantify calling based on NAAMP guidelines and included 4 categories: **0** =none heard calling, **1** = individual anurans detected, calls not overlapping, estimate number of individuals calling, **2** = individual calls distinguishable, but calls are overlapping, estimate number of individuals calling, **3** = individuals too numerous to count, chorus is constant and overlapping. At each survey station, we also recorded weather variables beginning of the 5-min period (air temperature [recorded from calling survey station] and water temperature [recorded at edge of water nearest calling station 1-2 cm below the water surface; both temperatures were recorded with mercury thermometers], pond pH, wind speed [Beaufort scale], estimated cloud cover, days since last rain, current precipitation [if any]), and number of cars that passed during the 5-min. survey. We also used a Massachusetts noise disturbance index to categorize ambient noise levels: **0** = no appreciable effect (*e.g.* owl calling), **1** = slightly affecting sampling (distant traffic, dog barking, one car passing), **2** = moderately affecting sampling (distant traffic, 2-5 cars passing), **3** = seriously affecting sampling (continuous traffic nearby, 6-10 cars passing), **4** = profoundly affecting sampling (continuous traffic passing, construction noise).

Data Analysis

To evaluate seasonal variation in anuran calling behavior, we used information-theoretic methods (Anderson *et al.* 2000) to estimate detection probabilities (p) and the proportion of wetlands occupied (Ψ) by each anuran species using the Occupancy Estimation routine in Program MARK (White and Burnham 1999), which was based on research by MacKenzie *et al.* (2002). We first determined encounter histories for each pond monitored with manual calling surveys during 19 10-day periods starting on 19 March and ending on 15 September. For this analysis, we were interested only in whether or not the species was detected during the 5-min calling survey at each pond and ignored NAAMP calling indices. This analysis permitted missing values when ponds were not sampled during a 10-day period. We first modeled the peak calling period for each species using the full model p_t, $\Psi.$, where t = time period. That is, we assumed detection probabilities varied among the 19 10-day survey periods and there was no temporal variation in wetland occupancy probabilities. To determine dates when peak calling occurred for each species, we used Akaike Information Criteria (AIC_c) values to compare among four models. AIC is information-theoretic methods that focus on providing a strength of evidence for an *a priori* set of alternative hypotheses, rather than a statistical test of a null hypothesis. AIC allows one to optimize model selection and estimation under a single theoretical framework (Anderson *et al.* 2000). The four models used were: model (1) p_t, $\Psi.$: we assumed there were 19 separate detection probabilities, one for each 10-day period, occupancy probabilities were constant; model (2) p_{peak}, $\Psi.$: we assumed two detection probabilities existed, one during all 10-day periods when the species was calling, and one during time periods when the species was not detected, occupancy probabilities were constant; model (3) p_{2peak}, $\Psi.$:, we assumed 2 levels of calling probabilities during 10-day intervals when the species was detected (a 'peak' period and a 'moderate' calling period), and detection probabilities were constant when the species did not call, occupancy probabilities were constant; and model (4) $p_{multipeak}$, $\Psi.$: we assumed detection probabilities varied among each 10-day period when a species was calling, and detection probabilities were constant during time periods when the species was not detected, occupancy probabilities were constant. We then used model averaging to estimate detection probabilities and proportion of wetlands occupied for each species (Anderson *et al.* 2000).

We were interested in determining how long surveys should be conducted to have a detection probability of >80% on days when the species was vocalizing. We used accumulation curves, which plot the percentage of surveys with at least one call within the each 1-minute segment and used only surveys when at least 1 call was detected during the entire 5-min. survey. All analyses were done using SPSS 8.0 (SPSS 1998). Significance was evaluated at $P < 0.05$.

We conducted a power analysis to evaluate the ability of calling surveys to predict anuran population trends using the program MONITOR (J. Gibbs, SUNY-Syracuse; http://www.mp2-pwrc.usgs.gov/powcase/monitor.html). We were interested in determining how many breeding ponds would need to be surveyed to detect annual population declines of 5% and 10% over a 10-year period for each of the species detected during call surveys. Power was considered sufficient at >80% (i.e. when we had at least an 80% chance of detecting a population decline), with an alpha level of 0.05. Inputs of this analysis were calculated separately for each species and included the mean and standard deviation for both the calling index and the number of individuals calling. We conducted the power analysis using both the mean call index, as well as indices based on presence/absence of calling.

Automated Recording Systems

Site Selection

To quantify diel variation in call activity levels, we used automated recording systems (ARS) (Peterson and Dorcas 1994) at 5 wetlands at Cape Cod NS during 2001. We used ARSs to monitor calling at six sites (see Appendix II for habitat types). Sites were not randomly selected, rather they were selected based on the presence of taxa of interest. We had 2 ARSs available to us (provided by R. Jung, Patuxent Wildlife Research Center), so we moved recorders among ponds because no pond had all species present. Two sites were vernal ponds (E9 and E4; sites where wood frogs and spring peepers were known to exist prior ARS placement) in Eastham, two were placed in Wellfleet, one at a vernal cranberry bog (Motel Bog; where Fowler's toads were known to exist in previous years), and another at a shallow kettle pond (Grassy Pond; where green frogs were known to exist), one was placed in Truro at the Upper Pamet River (where both green frogs and bullfrogs were known to exist), and one was placed at a flooded dirt road in Provincetown (Hatches Harbor Dike Rd. adjacent to a cranberry bog) that had a high probability of detecting spadefoot toads.

Sampling Frequency and Sampling Unit

Each ARS was activated 24 hr per day and recorded for 30 sec every 30 min. A 90-min tape lasted about four days and tapes had to be changed every 45 hr. We monitored 2 vernal ponds in Eastham from 26 March through 31 July (Pond E9, 3,330 30-sec recordings) and 26 March to 23 April (Pond E4, 882 30-sec recordings), where wood frogs and spring peepers were known to breed. We monitored Motel Bog in Wellfleet from 28 April to 17 June (2,410 30-sec recordings). Finally, we monitored a site on the Upper Pamet River in Truro from 5 to 31 July (1,122 30-sec recordings), which had breeding green frogs and American bullfrogs, and a series of small puddles on a flooded dirt road in Provincetown (Hatches Harbor Dike Rd., adjacent to a cranberry bog) from 13 to 20 July (288 30-sec recordings) where we had observed adult eastern spadefoot during a rainstorm in early July 2001. Data transcription involved tape playback and recording the maximum calling index (NAAMP scale) for each anuran species for each 30-sec increment. Each 90-min tape took about 100 min to transcribe.

Each site also had a remote temperature/relative humidity gauge (± 0.3 ^{o}F accuracy and 0.05 ^{o}F resolution) and a rain gauge (0.01 mm collectors). Each data logger was fastened to a tree without a rain guard under a relatively closed canopy nearby the study pond, and the rain gauges were placed adjacent to those trees. The temperature and relative humidity were recorded at 10-min intervals.

Data Management

Data transcription involved tape playback and recording maximum calling indices (0-3 scale) for vocalizing male anurans at 30-second increments every 30 minutes. We recorded site, date, time, species, maximum calling index, precipitation (if heard) and ancillary data (i.e. sounds of birds, cars, people, dogs) for each 30-second interval. Temperature, relative humidity, and precipitation data were downloaded from each data logger approximately once per month and transferred to our base computer.

Data Analysis

To compare diel variation within and among species, we used circular statistics software (Oriana for Windows, Kovach Computing Services, Wales, United Kingdom, http://www.kovcomp.co.uk/oriana). Circular statistics are based on a von Mises distribution, which uses a unimodal distribution comparable to a normal distribution for linear data. Because NAAMP suggests observers start surveys 30 min after sunset, we first converted all observations to minutes after sunset based on data from the U.S. Naval Observatory (Washington, DC). We used a Rayleigh uniformity test (Batschelet 1981) to determine if anuran calling intensity was uniformly distributed across a 24-hr period. Probabilities < 0.05 suggest that the species showed evidence of a preferred time period for calling. We used Watson's F-test to compare calling chronology among ponds for individual species and to compare between species. We used Watson F-test to compare the lengths of the mean vector for each sample with that for the pooled data of the two samples. Watson's F statistics is the equivalent to Fisher's variance ratio statistic used in linear statistics.

To determine if water or air temperatures affected calling, we used temperature data collected during manual call surveys. For each species, we used only survey data collected during a species 'peak' calling period based on information-theoretic methods listed above. We used Mann-Whitney tests to compare air and water temperatures between surveys without detection and surveys with detections.

Tadpole Dyeing

Site Selection

Trapping using minnow traps (Gee's Minnow Traps, model G-40, 0.63cm mesh size, 43cm long by 22cm wide) in combination with tadpole dyeing, was attempted as a means of estimating larval population size of green frogs at deep kettle ponds. Minnow traps were in placed in one pond (W20) for a 3-day period and checked every 24 hours. All captured tadpoles were placed in a 19-liter (L) bucket and brought to the lab for experimental dyeing. Previously, researchers have used dyes to mark tadpoles (Herreid and Kinney 1966; Guttman and Creasy 1973; Travis 1981; Sinsch 1997). Using a modified protocol based on research by Jung *et al.* (2002), we experimented with using dyes as a marking technique to quantify population sizes for green frogs. Herried and Kinney (1966) stained wood frog tadpoles with 0.05% neutral red dye for 30 minutes and animals retained the mark for up to seven days, while Guttman and Creasey (1973) dyed green frogs with 0.002-0.004% concentrations for up to 3 hours with marks remaining visible for up to 10 days. Finally, marks on *Bufo calamita* were retained for at least 24 hours when immersed in 0.005% red dye for 15 minutes (Sinsch 1997).

Sampling Frequency and Sampling Unit

We used lower concentrations and shorter immersion times in dyes because recent research suggests some mortality at higher concentrations and longer immersion times in dyes (Jung *et al.* 2002). Experiments were conducted in the laboratory using neutral red dye (Fisher Scientific, N129-25). Dyeing experiments took place on 23, 24, 26, and 27 May. Tadpoles of *R. clamitans* were exposed to neutral red dye at one concentration (either 0.04, 0.02, 0.001, 0.002, 0.004, 0.0001, or 0.0002%) and exposure times of 10 or 20 minutes to monitor effects on growth and survival. The objective of this research was to develop a dye that would stain tadpoles in the field for at least 6 hours. The experimental design consisted of 15 19-liter (L) plastic buckets, 10 buckets with 4 dyed tadpoles each and 5 buckets with 4 undyed tadpoles. We placed 20 tadpoles of each species in 1 L natural pond water with dye. Tadpoles were then placed in buckets to monitor growth and survival for five days following exposure to dye. Tadpoles were fed 10% 3:1 Purina Rabbit Chow: TetraMin Fish Food.

Data Management

Stain longevity and visibility (if the animal was stained, how visible the stain was and which body part retained the stain), as well as tadpole health (i.e., lethargic, moribund, alive, abrasions present) was assessed and recorded 3, 6, 12, and 24 hours after exposure to dyes. Mass (to nearest 0.01 g) at the beginning and end of the experiment was measured for each group. We also recorded tadpole mortalities at 24-hour intervals.

Data Analysis

Percent mortality and dye retention on individuals (clearly dyed or not) were plotted against dye concentration and exposure time for each time interval (3, 6, 12, 18, and 24 hours) after initial exposure to dye.

Dip-netting

Site Selection

Researchers have previously used dip net counts to quantify amphibian community structure and determine salamander larvae and frog tadpole abundance (Shaffer *et al.* 1994). Yet, results from dip net surveys can be highly variable, biased toward certain species or life stages, and often not correlated with actual population size (see Jung *et al.* 2002). To determine if dip-netting is a powerful index of monitoring spotted salamander, Fowler's toad, and pickerel frog larval populations, we used D-frame dip nets to sample eight vernal ponds (W18, E4, E9, E10, T14, T15, P15 and P16), the Upper Pamet River, and two dune slack ponds (P6 and P13).

Sampling Frequency and Sampling Unit

All ponds were sampled with dip nets (5mm mesh, 40cm by 30cm net size) at least once from 29 May-8 June. Sampling consisted of 50 1-m long sweeps throughout the pond with Cummings aquatic D-frame nets.

Data Management

Data collected during dip net surveys consisted of site, date, start and end times, sweep number, quantity of each amphibian species present per sweep, type of invertebrates present, locality of sweep, and plant and debris types present where sweep occurred. We also sampled two productive spotted salamander ponds (E4, E9) a second time (24 sweeps per pond) to determine if we might have missed small larval *A. maculatum* during the first round of sampling.

Voucher Specimens

Identification of adult and juvenile amphibian to species on Cape Cod NS generally is not difficult, as there are relatively few species on the Cape. In addition, the species that do exist on the Cape are readily distinguished based on key characteristics (see our website for identification criteria; www.uri.edu/cels/nrs/paton; accessed on 15 September 2002). Identifying the larvae can be more difficult, it often is based on oral disc morphology (Orton 1952; Altig 1970). Therefore, we collected voucher specimens for larvae captured during dip-netting and minnow trapping when species identification was uncertain. Specimens were placed in 10% buffered formalin in glass specimen jars and are currently housed at P. Paton's lab at the University of Rhode Island.

Data Analysis

For dip net samples, we calculated the mean number of individual larvae captured per 1-m long dip net sweep. We used these data, mean number of larva per dip net sweep, to compare to maximum wetland area (m^2) using linear regression. Dip-netting data were also used to compare the relationship between larval density (number of individuals per sweep) to egg mass counts; we used linear regression to evaluate this relationship.

Egg Mass Counts

Based on prior monitoring efforts for amphibian populations in southern Rhode Island (Klemens 1993, Crouch and Paton 2000), there are two species whose populations could potentially be monitored at Cape Cod NS using egg mass counts: wood frogs (*Rana sylvatica*) and spotted salamanders (*Ambystoma maculatum*). These two species are among the earliest breeding species in southern New England, with wood frogs ovipositing during March and spotted salamanders from mid-March to mid-April (Portnoy 1990; Klemens 1993; Paton *et al.* 2000; Paton and Crouch 2002, Crouch and Paton 2002). Because embryo hatching rates are temperature dependent in amphibians and ponds are cold in the early spring (10-15 °C; Portnoy 1990), both species take 3-4 weeks for eggs to hatch. In contrast, egg masses of most other pond-breeding amphibians in southern New England hatch in <5 days (*e.g.*, green frog [*Rana clamitans*], pickerel frog [*R. palustris*], Fowler's toad [*B. fowleri*], eastern spadefoot toad [*Scaphiopus holbrookii*] Klemens 1993) or eggs are laid singly under leaf litter (*e.g.*, spring peeper [*Pseudacris crucifer*]; Klemens 1993) making detection of egg masses of most species extremely difficult.

During mid-March to mid-April, wood frogs tend to oviposit egg masses in communal aggregations at the north end of the pond attached to woody vegetation in shallow water, although this can be highly variable (Crouch and Paton 2000, Egan 2001). Spotted salamanders tend to deposit their egg masses more uniformly across pond bottoms, in slightly deeper water than wood frogs, although again this can be highly variable (Egan 2001). Based on prior research, we know there is a 1:1 relationship between the number of wood frog egg masses detected in the pond and the number of females that immigrate to a breeding pond, therefore egg mass counts can be a powerful index to monitor wood frog populations (Crouch and Paton 2000). Female spotted salamanders can lay one or more egg masses within the same breeding pond in the same breeding season (P. Paton, unpubl. data), therefore using egg mass counts as a population estimator can be less accurate.

Site Selection

We assessed the feasibility of using egg mass counts as an index to assess breeding population size by quantifying: (1) the amount of time it takes to survey ponds, (2) the number of egg masses found in ponds, (3) the phenology of oviposition, and (4) the characteristics of deposition sites (*e.g.*, water depth at deposition site, maximum water depth of pond, water temperature and pH, vegetation mass is attached to, quadrant of the pond, and water clarity). We selected nine ponds in Wellfleet and Eastham to conduct egg mass counts that coincided with ongoing hydrological monitoring programs (Martin *et al.* 1993), and historical egg mass counts (some ponds in the park [E01, T01, T14, T15, W01, and W07] have been monitored for five years or more). In addition, ponds selected for egg mass counts overlapped with calling surveys, that is egg mass counts were conducted at ponds where weekly calling surveys were also conducted.

Sampling Frequency and Sampling Unit

Each pond was surveyed 4 times from 28 March through 2 May. The selected ponds were as follows E1, E3, E4, E9, E10, T14, T15, W7 and W18 (see Appendix I & II for detailed descriptions of these ponds).

Data Management

Data collected during egg mass surveys included species, locus number (egg masses within 1 m of each other were consider a locus), number of egg masses and, in the case of spotted salamanders, type of mass (clear or white), mass stage, egg mass and water depth, and substrate attachment. Also start and end time, air temperature, water temperature, water clarity (Secchi disc), maximum water depth of the site, and weather conditions (sky and wind codes) were recorded during each survey.

Data Analysis

We calculated the total number of egg masses detected during each survey and the maximum count at each pond for the season. We used linear regression to compare maximum egg mass counts to wetland size (m^2). We also quantified the physical characteristics of each pond and egg mass loci (we use the term loci to describe communal egg mass aggregations, as female wood frogs and spotted salamanders will deposit egg masses in aggregations or solitarily; Egan 2001). We calculated mean egg mass depth (distance from upper surface of egg mass to pond bottom), pond quadrant, attachment substrate, and the proportion of egg masses that were either clear or milky white (only for spotted salamanders).

Nocturnal Road Surveys

Site Selection

We conducted nocturnal road surveys along selected routes, as another means to sample the spatial distribution of amphibians at Cape Cod NS. We surveyed roads from Eastham to Provincetown. The roads we surveyed in each town included: Eastham: Rte 6, Cable Road, Doane Road, Ocean View Road, Brackett Road, Nauset Road. Wellfleet: Rte 6, Headquarters Fire Roads, Ocean View, Lecount's Hollow, Long Pond, Cahoon Hollow, Gross Hill, and Gull Pond Roads. Truro: Rte. 6, Prince Valley, North Pamet, South Pamet, and Coast Guard Roads. Provincetown: Rte. 6. Race Point, Province Lands, and Hatches Harbor Dike Roads depending on time of year. The function of the nocturnal road surveys was to (1) determine locations of seasonal migratory pathways of spotted salamanders; (2) document new locations, breeding areas and migratory pathways of spadefoot toads, and (3) document incidental encounters of amphibians. We recorded snout-vent length and weight for most spadefoot toads we encountered. Gender of adult spadefoot toads was determined based on the presence of nuptial pads on the front digits of males, large individuals without nuptial pads were assumed to be females.

Sampling Frequency and Sampling Unit

Nocturnal road surveys were only conducted during nights 20:00-04:00 during or just after heavy rainstorms or thunderstorms in spring and summer (21 and 22 March, 4 and 6 April, 1 and 28 May, 2, 12, 17 and 18 June, and 1 July). During road surveys, we generally had 1 driver and 1 observer in a vehicle. We would drive at approximately 5-10 mph on the right hand side of the road with emergency flashers on to increase our visibility to other traffic on the road. During surveys from March-May, there was little traffic on side roads, so these surveys were not too dangerous. In June and July, traffic levels increased and road surveys were difficult, particularly on well traveled roads such as Route 6. When an amphibian was observed, we would pull the vehicle to the right hand side of the road, and the observer run to capture the animal, using a flashlight to find the animal. Most individuals stayed in the middle of the road if vehicle headlights were on the animal. Once captured, individuals were identified to species and measurements taken of spadefoot toads. Odometer readings were recorded for each capture location, and capture sites were revisited at a later date to calculate capture locations using a GPS. Observers wore reflective vests and flashing reflective belts to increase our visibility to oncoming traffic. Even though we were driving a federal GSA vehicle, we were often stopped by local police and park rangers during surveys.

Data Management

For each individual captured or seen during any survey, the following data were recorded (when possible): date, location (UTM coordinates based on a global positioning system, [GPS]), behavior, air and water temperature, species, age class (metamorph, juvenile, or adult), sex, reproductive condition, snout-vent and total length (for caudates, to the nearest 1 mm with a ruler), mass (to nearest 0.1 g with digital scale). After appropriate data were recorded, individuals were placed off the side of the road in the direction they were determined to be moving when first encountered.

Data Analysis

Global Positioning System (GPS) UTM coordinates were recorded for all individuals located on nocturnal road surveys with a 12-channel Garmin GPS III Plus (uncorrected accuracy 5-10 m). *B. fowleri* and *S. holbrookii* distribution maps were created using Arcview 3.2 and Massachusetts digital county overage maps.

Adult Spotted Salamander Trapping

Site Selection

Minnow traps (Gee's Minnow Traps, model G-40, 0.63cm mesh size, 43cm long by 22cm wide) were used to estimate population sizes of spotted salamanders and red-spotted newts. We selected three ponds for protocol development, which were ponds where calling surveys, egg mass counts, and dip net surveys were conducted. In March, trap deployment dates were dependent upon the location of immigrating adult spotted salamanders located on nocturnal road surveys.

Sampling Frequency and Sampling Unit

Upon location of the first immigrants to breeding ponds (24 and 30 March), traps were deployed for nine days at W7, six days at E9, and six days at T15. We placed 22 traps at W7, 10 at E9 and 10 at T15. The number of consecutive trapping days was chosen to reduce confidence intervals in population estimators, while the number of traps set was arbitrarily based on how many the observers it was felt were sufficient to sample the entire site. Traps were checked every 24 hours after initial deployment.

Data Management

Captured salamanders were toe clipped (to avoid double counting newly captured individuals), and captured newts were cohort-marked by clipping the distal end of the tail (<2mm.). Snout vent length (SVL), total length (TL) (both to the nearest mm), mass (g), age, sex, and breeding condition (gravid or non-gravid) were recorded for each individual. We initially had hoped to test the feasibility of using pattern mapping as a tool to recognize individuals, (visually and on film; Loafman 1991), however due to logistical constraints were unable to complete this aspect of the study. Additional data collected during each visit included start and end times, site name, air temperature, water temperature, and pond pH.

Data Analysis

We used Schnabel-Schumacher and Lincoln-Peterson mark-recapture models to estimate population sizes of minnow trapped *A. maculatum* populations, with 95% confidence intervals (CI) calculated for all estimates (Krebs 1999).

Larval Spotted Salamander Trapping

Site Selection

Sites selected for larval trapping were the same as those used for adult trapping (E9, T15, and W7) in order to test the effectiveness of this technique in correlation with adult trapping and egg mass counts. The effectiveness of minnow trapping (Gee's Minnow Traps, model G-40, 0.63cm mesh size, 43cm long by 22cm wide) as a means of estimating larval *A. maculatum* population size was later tested by re-trapping in three ponds (E4, E9 and E10) to assess capture rates of larval spotted salamanders.

Sampling Frequency and Sampling Unit

Spotted salamander metamorphs tend to emerge from breeding ponds in July and August in Rhode Island and Connecticut (Klemens 1993; Paton and Crouch 2002), therefore we began larval trapping on 10 July. Each of the three sites was sampled for three consecutive days.

Data Management

Captured larval spotted salamanders were cohort-marked by clipping the distal end of the tail (<2mm) in order to differentiate initial captures from recaptures. During each survey, we recorded start and end times, site name, air temperature, water temperature, water temperature, and pond pH.

Data Analysis

Schnabel-Schumacher and Lincoln-Peterson population estimation (Krebs 1999) was used for mark-recapture analysis of minnow trapped larval *A. maculatum* populations, with 95% confidence intervals (CI) were calculated for all estimates.

Transect Surveys in Ponds

In order to sample tadpoles of green frogs and bullfrogs, we field tested a transect survey technique. These two species require, in general, 1-2 years to metamorphose and therefore are limited to breeding in ponds with semi-permanent to permanent hydroperiods (Paton and Crouch 2002). Because many of deep kettle ponds on the Cape tend to be very large, dip-netting is probably not a valid technique to assess these populations. Therefore we conducted transect surveys in several large, relatively clear deep kettle ponds within the Seashore. While this technique may not be appropriate in monitoring slight fluctuations in population sizes, they could be useful for detecting gross population changes or malformations of individual morphology at ponds that are heavily utilized for recreational purposes. During pilot surveys to the Seashore in 1999, we found lethargic, depredated bullfrog tadpoles in Gull Pond, with their tails completely consumed (presumably by fish). These animals were easy to detect in the clear water, therefore we thought walking transects might be a useful technique to survey amphibians.

Site Selection

Three deep kettle ponds on the Seashore (Duck Pond, Gull Pond, and Kinnacum Pond, all in Wellfleet) were selected to test this technique. These ponds were selected because they had green frog and bullfrog tadpoles and the ponds were relatively clear (secchi disc readings of over 5 m), thus we could identify tadpoles to species and count individuals resting on the pond bottom.

Sampling Frequency and Sampling Unit

Each site selected was sampled with 3 100-m transects, with the exception of Gull Pond in which 6 transects were conducted. The number of transects selected per pond was a function of the perimeter length of the pond as not to overlap any transects and to get a fairly good idea of the distribution around the entire pond. Sites were surveyed during the middle of June (10 June at Duck Pond, 20 June at Gull Pond and Kinnacum Pond) in early and late-afternoon hours on clear days when glare off the surface of the water was minimal and visibility was high. The first 100-m transect was located at a random spot around the pond perimeter, with transect length determined with a laser range finder. Each subsequent transect at that site was started 100-m beyond the terminus of the previous transect. During transect surveys, we walked in the water at a constant distance from the pond shoreline (no farther than 10 m from the shore) and walked at a constant rate of speed. We recorded all tadpoles detected within 3 m on either side of the transect.

Data Management

The start and end times of each individual transect, and the entire survey, were recorded. Also a running count of the number of tadpoles encountered of each species was recorded for each individual transect at each site on field data sheets.

Data Analysis

The mean number (± SD) of tadpoles by species per transect was calculated for each site, as well as the mean (± SD) amount of time required to complete each transect.

Size Characteristics of Individual Taxa

Linear regression was used to quantify relationships between snout-vent length (SVL) and mass in males and females in *A. maculatum* populations, and for all documented (male and female collectively) individuals of *Scaphiopus holbrookii*. Linear regression, scatter plot and a frequency histogram were used to show relationships between SVL and body mass. Descriptive statistics for morphological characters (i.e. SVL, TL and mass) were calculated using SPSS.

Estimating Labor and Descriptive Statistics

We evaluated the economic feasibility of using particular field methodologies (i.e. dip-netting minnow trapping, egg mass counting, tadpole dyeing, road surveys) by calculating the mean number of person-hours (labor) involved with individual sampling activities.

RESULTS

Manual Anuran Call Surveys

Species Detection Probabilities

Modeled detection probability estimates represent the probability of surveying a pond once during a species' peak period and detecting that species. These models suggest that within a species' peak survey window, most species have weeks with moderate detection probabilities and weeks with relatively high detection probabilities (see Fig. 1), while occupancy probabilities were constant over time. Based on model averaging, *B. fowleri* had the lowest mean detection probability ($\bar{x}$ = 0.469 ± 0.095 [SE]) during peak calling periods (Tables 2 and 3). *R. sylvatica, R. palustris,* and *R. catesbeiana* had moderate detection probabilities (0.62 to 0.74), while *P. crucifer*, and *R. clamitans* had the highest detection probabilities during peak calling periods (0.81; Fig. 1). *R. sylvatica* and *R.*

palustris had limited distributions at Cape Cod, these species were estimated to occur in 8.4% and 14.3%, respectively, of the wetlands we sampled (Table 2 and 4). *B. fowleri* and *R. catesbeiana* were estimated to occur in about one-third of the wetlands and *R. clamitans* were in almost 60% of wetlands. *P. crucifer* was the most widely distributed species at Cape Cod, occurring in >90% of all wetlands in the study area. We detected wood frogs on 75% of call surveys during their peak period, but were only able to conduct 4 surveys during their peak period, thus we were unable to include in estimation models. We never detected eastern spadefoots during systematic call surveys.

Table 2. Estimates of detection probabilities (p) and wetland occupancy probabilities (Ψ) for six species of anurans at Cape Cod NS based on models presented in Table 2.

Species	**Detection probability**		**Proportion of wetlands occupied**	
	$\bar{x}$	**SE**	$\bar{x}$	**SE**
B. fowleri	0.469	0.095	0.390	0.095
R. catesbeiana	0.696	0.073	0.320	0.090
R. clamitans	0.805	0.038	0.598	0.091
R. palustris	0.749	0.098	0.143	0.059
R. sylvatica	0.619	0.333	0.084	0.065
P. crucifer	0.805	0.034	0.938	0.043

Table 3. The best models to estimate detection probabilities (p) and wetland occupancy probabilities (Ψ) of 6 anuran species using manual call surveys. The bias-corrected Akaike Information Criteria (AIC_c), the difference in AIC_c values between the *i*th model and the model with the lowest AIC_c value (Δ_I), the Akaike weights (w_i) for the set of models that represent over 90% of Akaike weights, and the number of parameters in each model (K_i) are presented.

Species	**Model**	**AIC_c**	D_i	**w_i**	**K_i**
B. fowleri	$\{p_{2peak}\ \Psi.\}$	124.2	0.00	0.997	4
P. crucifer	$\{p_{2peak}\ \Psi.\}$	315.3	0.00	0.998	4
R. palustris	$\{p_{2peak}\ \Psi.\}$	70.5	0.00	0.847	4
	$\{p_{multipeak}\Psi.\}$	74.6	4.09	0.109	7
R. sylvatica	$\{p_{peak}\ \Psi.\}$	25.4	0.00	0.601	3
	$\{p_{2peak}\ \Psi.\}$	26.26	0.83	0.397	4
R. clamitans	$\{p_{2peak}\ \Psi.\}$	231.3	0.00	1.000	4
R. catesbeiana	$\{p_{peak}\ \Psi.\}$	92.69	0.00	0.542	3
	$\{p_{2peak}\ \Psi.\}$	93.04	0.35	0.455	4

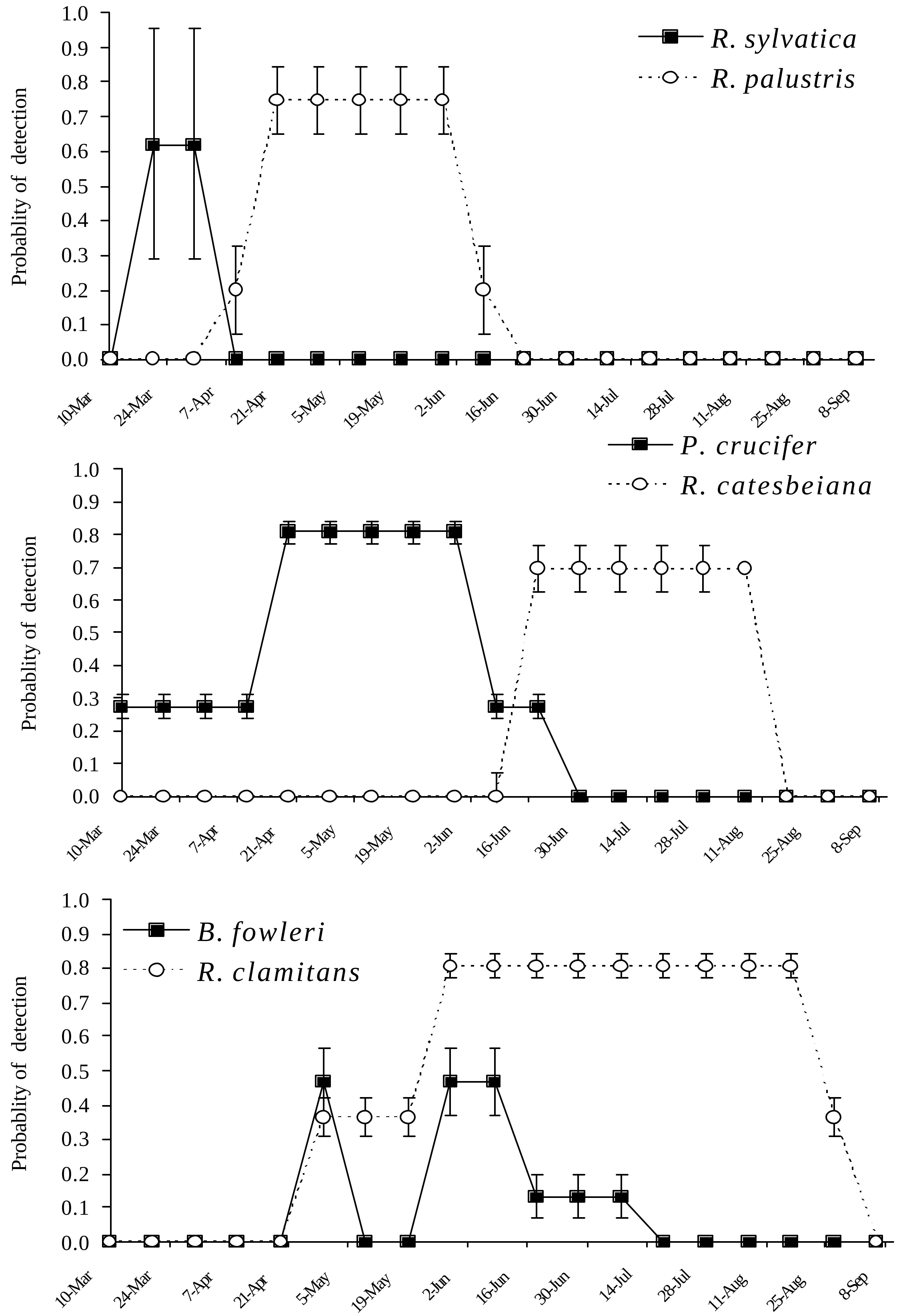


Figure 1. Seasonal variation in detection probabilities (mean ± SE) of calling anurans at Cape Cod National Seashore, Massachusetts based on models presented in Table 3.

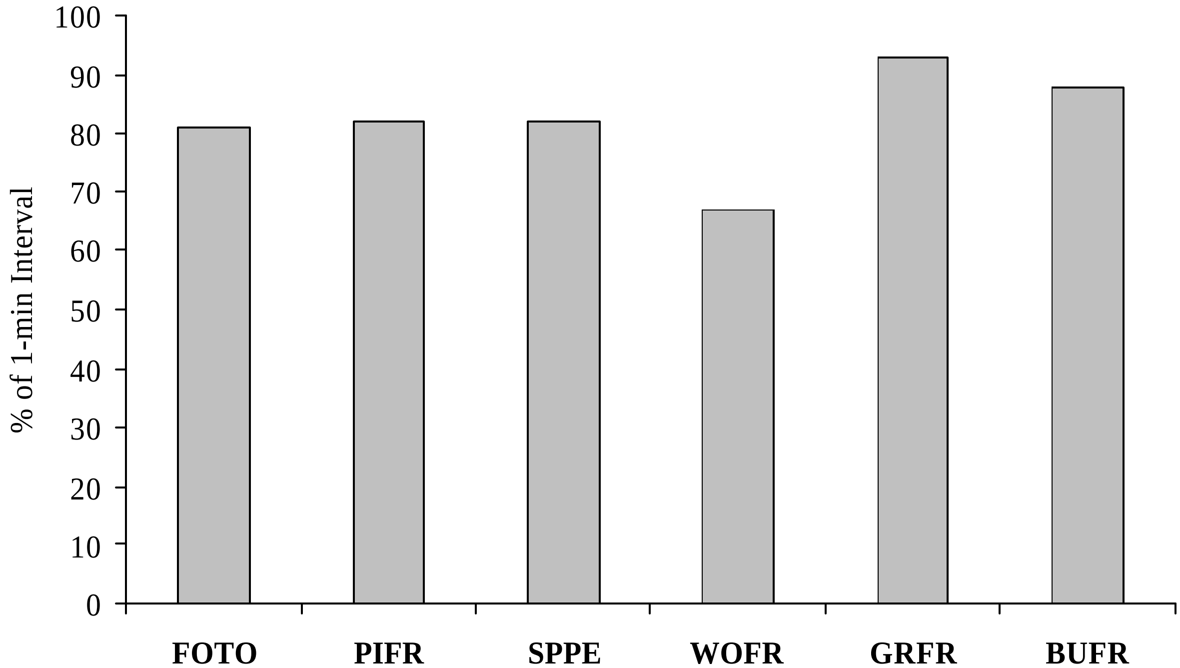


Figure 2. Percentage of 1-min intervals within the 5-min interval during manual anuran call surveys that had detections; this analysis used only surveys that had at least 1 detection during the 5-min survey. FOTO=fowler's toad, PIFR=pickerel frog, SPPE=spring peepers, WOFR=wood frog, GRFR=green frog, BUFR=American bullfrog

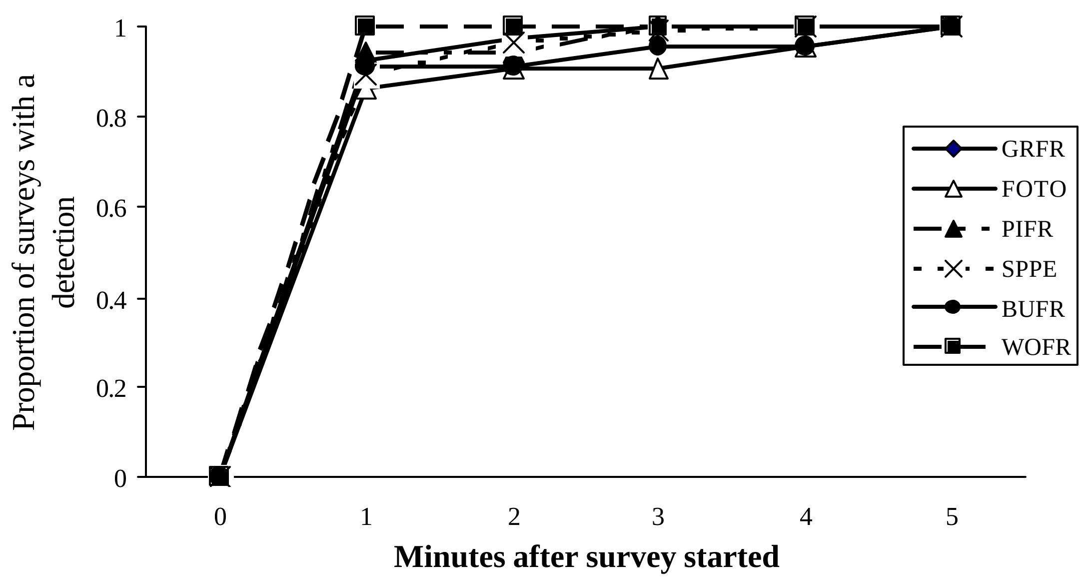


Figure 3. Accumulation curves showing probability of detecting a calling anuran within the 5-min. survey period; only for surveys when the species was detected. See codes on Fig. 2.

Within call surveys, green frogs vocalized most frequently, as they were detected on 93% (n = 360) of 1-min segments within surveys that had at least 1 detection (Fig. 2). Wood frogs vocalized less frequently during call surveys than all other species (67%, n = 15), and bullfrogs, spring peepers, pickerel frogs and Fowler's toads frequently vocalized, calling on 88% (n = 115), 82% (n = 590), 82% (n = 100) and 81% (n = 110) of 1-min intervals, respectively. Based on accumulation curves (Fig. 3), all species were generally detected within 1 min after initiation of the survey during surveys when they vocalized.

Table 4. Sites where each species was detected at least once during anuran call surveys at Cape Cod NS in 2001.

Pond	Bullfrog	Fowler's toad	Green frog	Pickerel frog	Spring peeper	Wood frog
Ballston Marsh	X	X				
Black Pond	X		X	X	X	
E4			X		X	X
E9			X		X	X
E15			X		X	
E16			X		X	
E18			X		X	
Grassy Pond	X		X		X	
Great Pond		X	X		X	
Gull Pond	X	X			X	
Herring Pond	X		X	X	X	
Kinnacum Pond	X	X	X		X	
Lily Pond Main		X	X		X	
Lily Pond South		X	X		X	
Motel Bog		X	X		X	
P5		X			X	
P6		X			X	
P8		X			X	
P13		X			X	
P16					X	
Pamet Bog	X		X	X	X	
Snow Pond		X	X	X	X	
T14			X		X	
T15					X	
Upper Pamet	X		X	X	X	
W7			X		X	
W17					X	
W18					X	
W20	X	X	X		X	

Seasonal variation in calling rates

Each species had a distinctive calling period, which extended over 8 days for *R. sylvatica* (28 March to 5 April), 52 days for *R. palustris* (17 April to 8 June), 55 days for *R. catesbeiana* (8 June to 2 August), 68 days for *B. fowleri* (2 May to 9 July), 100 days for *P. crucifer* (19 March to 27 June), and 119 days for *R. clamitans* (2 May to 29 August) (Fig. 1). For all anuran species we monitored, we found distinctive time periods when calling activity levels peaked and detection probabilities increased (Table 2). Based on the best model for each species, we estimated peak calling periods of 20 March to 8 April for *R. sylvatica*, 19 April to 7 June for *R. palustris and P. crucifer*, 29 April to 17 June for *B. fowleri*, and 8 June to 6 August for *R. catesbeiana*, 29 May to 26 August for *R. clamitans* (Fig. 1). Variation in peak call periods occurred among species, sites, and routes at Cape Cod NS (see also Appendix III, IV, and V).

Automated Recording Systems

Diel variation in call chronology

We detected 5 anuran species with ARSs at Cape Cod, but never detected *R. palustris* or *S. holbrookii*. All 5 species exhibited a peak time period of calling within a 24-hr period (Rayleigh test of uniformity; $P < 0.01$). *R. sylvatica* was the only species that had a high probability of calling during afternoon and early evening hours (Fig. 4). We found no difference in calling chronology of *R. sylvatica* between ponds (Pond E9: $\bar{x}$ = 1820 hr, 99% CI = 1603 – 2037 hr; Pond E4: $\bar{x}$ = 1947 hr, 99% CI = 1815 – 2119 hr; $F = 2.39$, $P = 0.12$). When we pooled data from both ponds, the mean peak calling time for *R. sylvatica* was 13 min after sunset (99% CI = 81 min before sunset – 106 min after sunset).

We detected no difference between the calling chronology of *P. crucifer* and *B. fowleri* ($F = 0.46$, $P = 0.50$). Both species reached peak calling intensity within 2.5 hr after sunset, with *B. fowleri* having a mean peak calling period 89 min after sunset (99% CI = 70 min – 108 min after sunset) and *P. crucifer* 157 min after sunset (99% CI = 142 min – 172 min after sunset; Fig. 4). *B. fowleri* called only from sunset to approximately midnight, whereas *P. crucifer* called throughout the night and sporadically throughout daylight hours. *P. crucifer* called most actively from 2000 to 2300 hr, with minor variation among ponds (Pond E: $\bar{x}$ = 2119 hr, 99% CI 2102 – 2136 hr; Motel Bog: $\bar{x}$ = 2216 hr, 99% CI = 2154 – 2239 hr; Pond E4: $\bar{x}$ = 2059 hr, 2031 – 2137 hr).

Both *R. clamitans* and *R. catesbeiana* reached peak calling later in the evening than the other 3 species, with mean peak calling intensity around 197 min (99% CI = 180 min to 215 min after sunset) and 237 min after sunset (99% CI = 204 min to 271 min after sunset), respectively (Fig. 4). There was a 36-min difference in the calling chronology of *R. clamitans* between Upper Pamet River ($\bar{x}$ = 0009 hr, 99% CI = 2325 – 0023 hr) and Pond E9 ($\bar{x}$ = 2333 h, 99% CI = 2313 – 2353 hr) ($F = 6.2$, $P = 0.01$), which we interpreted as more of a statistical difference than a biological meaningful difference. There was no difference between the mean peak calling period for *R. catesbeiana* at Upper Pamet River ($\bar{x}$ = 2354 hr; 99% CI = 2325 − 0023 hr) and *R. clamitans* at Upper Pamet River (F = 0.68, P = 0.41) or Pond E9 (F = 1.51, P = 0.22).

Call Intensity

Spring peepers called the most intensely as well as the most frequently (Fig. 5). Peepers usually had loud choruses, often with an index value of (3). In contrast, bullfrogs and wood frogs called the least intensely, with bullfrogs never reaching a full chorus (index value 3) and wood frogs reaching a full chorus on only one occasion. Pickerel frogs reached a full chorus on two occasions and Fowler’s toads and green frogs occasionally were encountered while in full chorus (see Appendix III, IV, and V).

Effects of Environmental Variation on Calling Behavior

For most species we monitored during manual call surveys, we found little evidence that air or surface water temperatures affected calling behavior (Fig. 6). However, there was a tendency for most species to be more sensitive to surface water temperature than air temperatures. While we documented three species (*P. crucifer*, *R. palustris* and *R. clamitans*) calling on evenings when air temperatures were below 8 °C (with *P. crucifer* calling when air temperatures were as low 1.7 °C), calling was almost always associated with water temperatures above 10°C (50°F). Only *P. crucifer* was recorded calling when water temperature was less than 10°C. This suggests that calling activity is determined primarily by water temperature, and that little calling occurs when water temperatures are below <10°C.

Labor Required for Calling Surveys and ARS Maintenance

One call survey route took roughly 2.5 hours (2.5 person-hours) for one observer to complete (mean = 134.6 min, SD = 49.5). Three routes per week were sampled, yielding 7.5 hours of labor per week for call surveys. Four days of data from a ARS (i.e., 1 tape) took three hours to transcribe and enter. At times, four ARS were deployed, resulting in approximately 12 hours of transcription and data entry per four days. Travel time was roughly two hours to change ARS tapes every two days (when 4 ARS units were deployed), resulting a total of 16 hours of labor per four days of ARS activity.

Power Analysis Based on a Species' Mean Call Index

Power analysis results varied among species. As would be expected, results suggests that more survey effort is required for species that vocalize less consistently during peak call periods than species that vocalize more continuously during peak call periods.

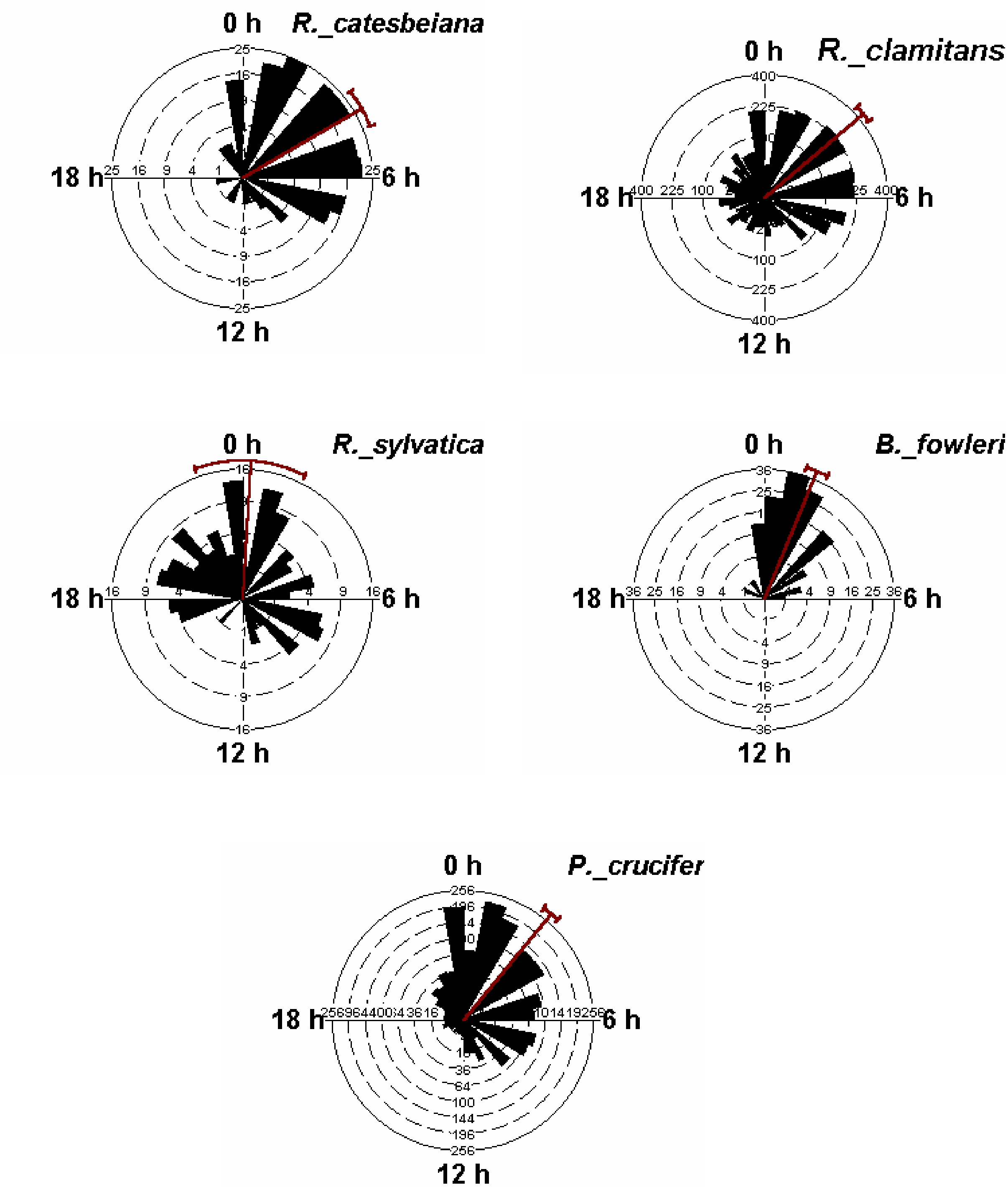


Figure 4. Hours after sunset that 5 species of anurans were detected with automated recording systems at Cape Cod National Seashore, Massachusetts. Frequency of detections (shown by numbers within circles) for a given time period is represented by area of the wedge. Mean time of peak calling (99% CI) is given by narrow line outside of largest circle.

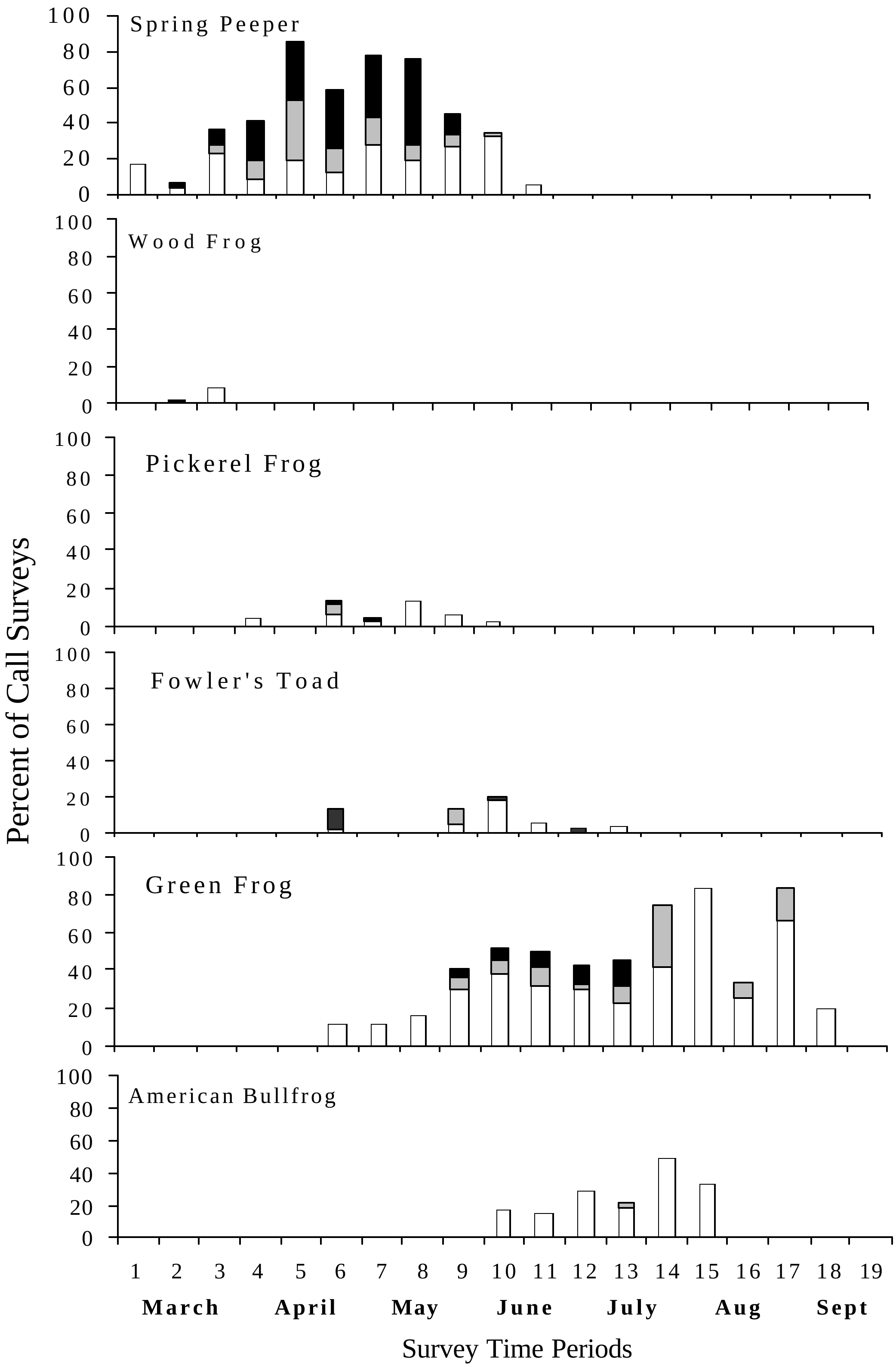


Figure 5. Seasonal variation in call intensity of anurans at the Cape Cod NS in 2001 based on manual call surveys. Calling index (1) = white bars, calling index (2) = gray bars, calling index (3) = black bars. Survey time periods: 1=Mar 10-19, 2=Mar 20-29, 3=30 Mar-8 Apr, 4=9-18 Apr, 5= 19-28 Apr, 6=29 Apr-8 May, 7=9-18 May, 8=19-28 May, 9=29 May-8 Jun, 10=9-17 Jun, 11=18-27 Jun, 12=28 Jun-7 Jul, 13= 8-17 Jul, 14=18-27 Jul, 15=28 Jul-6 Aug, 16=7-16 Aug, 17=17-26 Aug, 18=27 Aug-5 Sept, 19=6-15 Sept.

Based on the power analysis for 28 sites we sampled during the 2001 field season, spring peepers and green frogs were the only species that would require minimal survey effort to monitor their long-term population trends. Each site where spring peepers were detected calling at least once this season would only have to be surveyed once per year for 10 years during the peak calling period and during prime calling conditions to detect a 5% and 10% increase and decrease in breeding populations of spring peepers and green frogs (Table 5).

Since Fowler’s toads call less consistently than spring peepers during peak calling periods, more survey effort is required to monitor their long-term population trends (Table 5). Call surveys would need to be conducted on 8 different occasions at 13 sites where Fowler's toads were heard this season during the species' peak calling period to detect a 5% and 10% increase and decrease in breeding aggregations (for 13 sites).

Table 5. Power to detect population declines and increases with 95% confidence over 10 years for five species of anurans at Cape Cod National Seashore using calling surveys. Power analyses were based on using call indices or presence/absence analysis. Number of surveys refers to the total number of surveys that would have to be conducted annually to reach power ≥0.80. Also shown is the peak call period for each species and the total number of sites where each species was detected during systematic call surveys.

Species	Peak call period	No. Sites	No. surveys	Total No. Station Visits	Population decline		Population increase	
					5%	10%	5%	10%
Call indices								
Spring peeper	4/17-5/24	28	1	28	0.96	1.00	1.00	1.00
Fowler's toad	5/2-6/21	13	8	104	0.80	1.00	0.96	1.00
Pickerel frog	5/1-5/30	5	10	50	0.85	1.00	0.96	1.00
Green frog	5/29-7/5	19	1	19	0.87	1.00	0.98	1.00
Bullfrog	6/13-7/5	8	4	32	0.90	1.00	0.97	1.00
Presence/absence								
Spring peeper	4/17-5/24	28	1	28	1.00	1.00	1.00	1.00
Fowler's toad	5/2-6/21	13	7	91	0.84	1.00	0.96	1.00
Pickerel frog	5/1-5/30	5	7	35	0.82	1.00	0.94	1.00
Green frog	5/29-7/5	19	1	19	0.94	1.00	1.00	1.00
Bullfrog	6/13-7/5	8	2	16	0.83	0.98	0.94	1.00

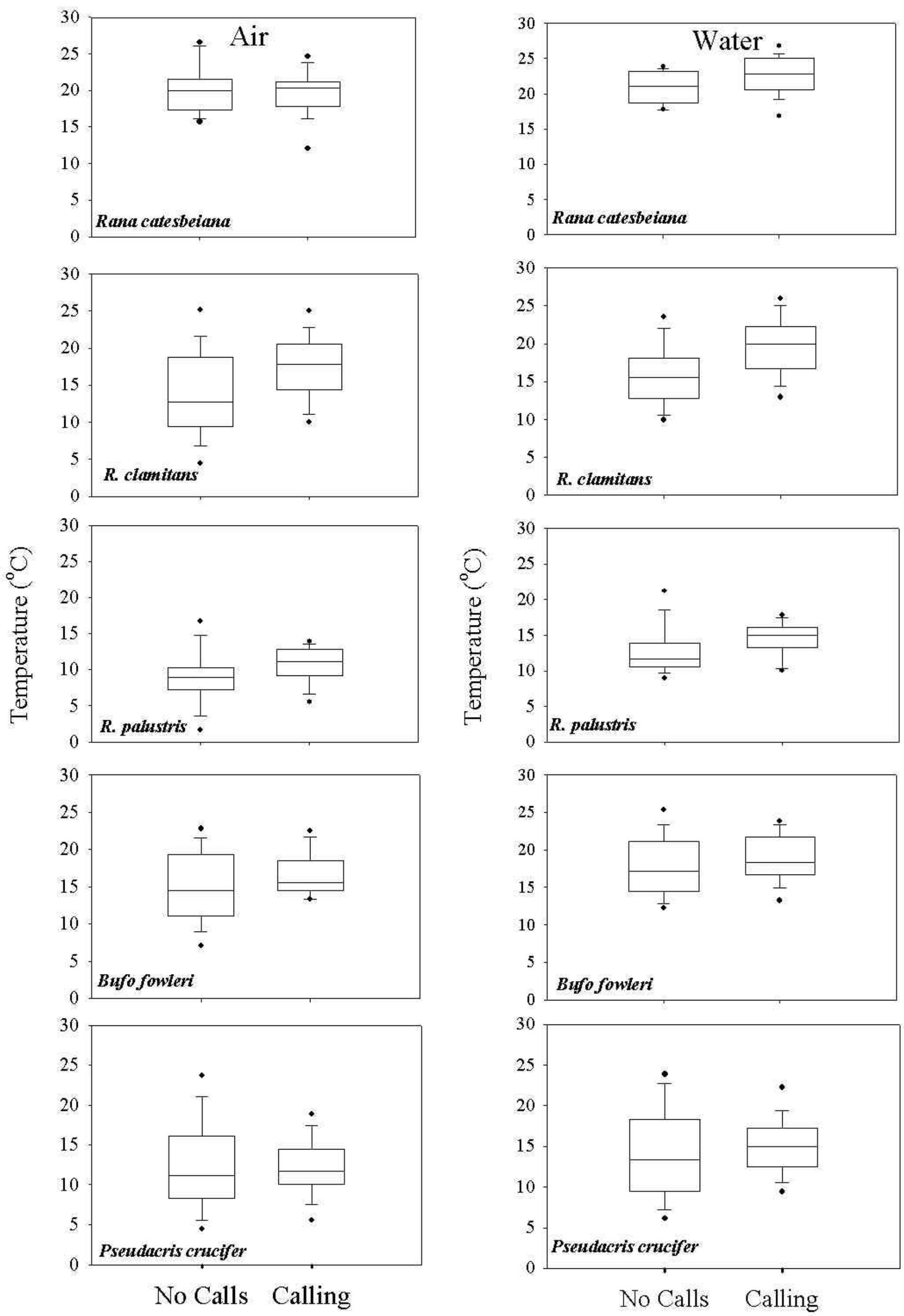


Figure 6. Effect of air and water temperature on probability of detecting calling anurans during manual call surveys at peak calling periods within Cape Cod National Seashore. Boxes bound the 25% and 75% quartiles with median in center of box, error bars are 10^{th} and 90^{th} percentiles, and dots represent 5^{th} and 95^{th} percentiles.

Like the Fowler's toad, pickerel frogs vocalized less consistently during their peak calling period than other calling anurans, and it would require substantial effort to detect changes in pickerel frog populations (Table 5). Power analysis of five sites where pickerel frogs were detected suggests that 10 surveys at each site during the species' peak calling period are required to detect a 5% and 10% increase and decrease in breeding aggregations

Bullfrogs would require call surveys on 4 separate occasions at the 8 sites they were detected calling during their peak call period for ten years to sufficiently detect significant population changes (Table 5). Bullfrogs vocalized more consistently than Fowler's toads and pickerel frogs, however calling indices of bullfrogs never reached a full chorus.

Power Analysis Using Species Presence/Absence

We also conducted a power analysis for each species using presence or absence of calling, instead of mean calling indices (Table 5). Generally, using a presence/absence approach required less surveying effort to detect changes in population size compared to using call indices. To adequately detect annual population decline of 5% (cumulative 37% decline over 10 years) the following effort is required (all surveys must be completed during species' peak and for 10 consecutive years): (1) Spring peeper (28 sites), one survey, (2) Fowler's toad (13 sites), seven surveys, (3) Pickerel frog (5 sites), seven surveys, (4) Green frog (19 sites), one survey and (5) Bullfrog (8 sites), two surveys during peak for 10 years (Table 5).

Tadpole Dyeing

Tadpole dyeing was tested and determined to be an impractical means of estimating larval population sizes of green frog. All concentrations that yielded little or no larval mortality did not effectively stain animals to validate use in the field (Table 6). Green frog tadpole dyeing was labor intensive and not practical under field conditions. Tadpoles that were submersed in neutral red dye for 10 and 20-minute intervals at all concentrations (0.0002%, 0.001%, 0.002%, and 0.004%) did not hold observable amount of dye three hours after initial submersion in the dye. These concentrations lightly stained hind limbs, dorsal and ventral caudal keels, cloacal tissue and the abdomen of *R. clamitans* larvae at three hours after submersion with no mortality, but stain was faint enough that it would be overlooked in the field by most observers. At three hours after dying, all dyed individuals retained a slight pink hue on ventral caudal keels and around cloacal tissue, but dye was only noticeable after extremely close examination of the individual. These concentrations produced no larval mortality. Concentrations that effectively stained larvae had a negative impact on larval survival.

Table 6. Results of experimental tadpole dyeing. Given are dye concentration, exposure time (minutes), hours after initial exposure, and whether or not the dye retained would be readily recognized in the field.

Dye Conc. (%)	Exposure time (min)	Hours after exposure	% Mortality	Dye retained?
0.0002	10	3	0	No
0.0002	20	3	0	No
0.0002	10	6	0	No
0.0002	20	6	0	No
0.001	10	3	0	No
0.001	20	3	0	No
0.001	10	6	0	No
0.001	20	6	0	No
0.001	10	12	0	No
0.001	20	12	0	No
0.002	10	3	0	No
0.002	20	3	0	No
0.002	10	6	0	No
0.002	20	6	0	No
0.002	10	12	0	No
0.002	20	12	1	No
0.002	10	18	0	No
0.002	20	18	1.5	No
0.004	10	6	0.5	No
0.004	20	6	0	No
0.004	10	8	0.5	No
0.004	20	18	1	No
0.004	10	24	0.5	No
0.004	20	24	1	No
0.04	20	24	25	Yes

Dip-netting

Dip-netting was most successful at ponds that contained large breeding populations of spring peepers (Table 7). Spotted salamander and pickerel frog larvae were not easily captured via dip-netting, thus this method should not be used to estimate larval density of *A. maculatum* or *R. palustris*. The highest capture rate for spotted salamander was 0.54 larvae per sweep at E10 (where hundreds of egg masses were documented), and pickerel frog larvae were only detected at the Pamet River. We found no significant relationship between size of the pond sampled and minutes required to sample these ponds, and a weak correlation between larval density and time required to sample ponds (R^2 = 0.143). This sampling method required between 102 and 144 min (3.4 and 4.8 person-hours) to complete 50 dip net sweeps at a site. Dip-netting prior to 10 July on Cape Cod NS is not feasible because larval *A. maculatum* are too small for dip net retention.

Table 7. Interpond differences in capture rates (mean number of individuals per sweep [± SD]) and frequency of captures (% of sweeps with a capture) of pickerel frog, spring peeper, and spotted salamander larvae during dip net sweeps at Cape Cod NS in 2001.

Species & Site	**Date**	**Capture rates (no. ±SD)**	**Frequency (%)**	**Total # sweeps**
Pickerel Frog				
E4	30 May	0	0	50
E4	22 June	0	0	24
E9	29 May	0	0	50
E10	27 June	0	0	24
W18	29 June	0	0	24
Grassy Pond	8 June	0	0	25
W7	27 June	0	0	24
T14	29 June	0	0	29
T15	29 June	0	0	24
Upper Pamet	1 June	0.08 ± 0.27	8	50
P5	31 May	0	0	50
P13	28 May	0	0	24
Spring Peeper				
E4	30 May	21.9 ± 15.8	100	50
E4	22 June	11.2 ± 5.8	100	24
E9	29 May	3.4 ± 3.4	82	50
E10	27 June	1.0 ± .0	67	24
W18	29 June	0	0	24
Grassy Pond	8 June	2.3 ± 3.1	72	25
W7	27 June	0.8 ± 1.3	38	24
T14	29 June	0	0	29
T15	29 June	0	0	24
Upper Pamet	1 June	0	0	50
P5	31 May	0	0	50
P13	28 May	1.0 ± 1.4	58	24
Spotted Salamander				
E4	30 May	0.02 ± 0.14	2	50
E4	22 June	0	0	24
E9	29 May	0.2 ± 0.8	6	50
E10	27 June	0.5 ± 0.66	46	24
W18	29 June	0	0	24
Grassy Pond	8 June	0.1 ± 0.33	12	25
W7	27 June	0.3 ± 0.46	29	24
T14	29 June	0	0	29
T15	29 June	0	0	24
Upper Pamet	1 June	0	0	50
P5	31 May	0	0	50
P13	28 May	0	0	24

Egg Mass Counts

Counting egg masses was an efficient method to monitor *A. maculatum* breeding populations. Individual searches of ponds took approximately 60 min (mean = 59.6 min, SD = 49.1), and ranged from 45-180 min to complete a survey. Weather variables affected our ability to complete the egg mass counts. Overcast skies produced a mirror-like glare on top of tannin-rich waters in vernal ponds of the Seashore, which made it nearly impossible to locate egg masses. Rain, ice and snow also made locating egg masses difficult. The most favorable weather conditions for egg mass counting were clear, calm days before noon because direct, overhead sunlight also cast a glare on ponds, which made finding egg masses difficult.

The largest pond surveyed (W7, 2107m^2) contained the most egg masses (488) and the most females (72 females captured, Schnabel-Schumacher estimate of 211.1 females). Female spotted salamanders are thought to deposit 1-4 egg masses per females (J.W. Petranka, pers. comm.), thus our estimate of the number of females may be accurate. W7 was relatively clear, with Secchi disk visibility of 36cm. E9 was the second largest and clearest pond surveyed (1357.9 m^2, Secchi disk visibility of 60 cm) with both egg mass counts and minnow traps, and 469 egg masses and 14 females were captured via minnow trapping. No marked females were captured therefore population estimation could not be accomplished. T15 was the smallest, darkest (Table 8) pond surveyed (112.5 m^2) and it also had the fewest number of egg masses present (42), and had the smallest population of breeding females (Schnabel-Schumacher estimation of 37.4 individuals).

Table 8. Total number of spotted salamander egg mass loci (i.e., locations with individual or aggregations of egg masses) detected in selected ponds at Cape Cod NS in 2001. Total number of egg mass loci, mean water depth (cm±SD) at egg mass, maximum water depth (cm) of the pond during the date of the Secchi disc measurement, mean depth to water surface (cm±SD) from egg masses, and water clarity as measured by Secchi disc depth (cm) are presented.

Pond	**Number mass loci**	**Water depth at egg mass (cm ± SD)**	**Max. pond water depth (cm)**	**Water surface to egg mass (cm±SD)**	**Secchi Disc (cm)**
E3	12	15.8 (4.0)	22	3.8 (3.3)	22
E4	57	19.3 (3.7)	32	6.4 (3.8)	32
E9	69	34.6 (8.2)	60	11.8 (6.0)	60
E10	31	29.3 (5.0)	38	12.3 (6.6)	38
T15	9	33.9 (14.8)	61	2.2 (2.3)	10
W7	90	39.8 (14.9)	105	6.1 (3.6)	36

At some ponds, we counted many more egg masses than during surveys conducted in previous years (Table 9). This exemplifies the need to survey sites in multiple years in order to assess the size of the breeding population.

Spotted salamander oviposition began in late March. At most sites masses began hatching in late April. The egg mass counts at each site surveyed varied each time the site was visited (Table 10). The highest egg mass counts occurred between 22 April and 1 May. Later in the year, finding and counting egg masses became increasingly difficult for three reasons; (1) masses became stained with tannins, thus becoming darkened and difficult to discern from dark substrate, (2) masses began to swell to a size that caused them to detach from attachment sites and sink to the bottom, and (3) pond foliage became increasingly thick and made navigation throughout ponds difficult and covered many open areas in which oviposition took place. White masses were more visible, and at all sites where egg mass counts took place, the clear to opaque-white egg mass ratio was high (Table 11). The clear mass:white mass ratio ranged from 3.28:1 to 210:1, and there was no obvious relationship between quadrant and number of masses deposited. The two largest concentrations of egg masses were in the north (22.9%) and east (16.7%) quadrants of the pond. However our data indicate that the entire pond needs to be surveyed to count all masses within a pond.

Depending on the particular pond, egg mass substrate/vegetation attachment varied (Table 11, Figure 7). A total of 1,977 egg masses were located. Only 139 (7% of all egg masses) egg masses were milky white, of which the majority were in only one pond (W7; Table 10). The pond floor was the least common attachment site (1% of total masses located were deposited on floor) *Calamagrostis spp.*, *Smilax spp.*, *Sparganium spp.*, and woody (including emergent and fallen deadwood) harbored a small percentage of total egg masses. *Scirpus spp.* was the attachment site for 14% of the total egg masses and *Decodon verticillatus*, *Dulichium arundinaceum*, and *Juncus spp.*, harbored the majority of all located masses. Woody vegetation (fallen deadwood or emergent woody) was used as an attachment site at two ponds, which were predominantly open water. *Juncus canadensis* was the dominant vegetation at the one pond where oviposition occurred and *Smilax*, *Dulichium arundinaceum* and *Decodon verticillatus* were dominant vegetation at ponds where this vegetation was used as oviposition sites. However, at E10 most egg masses were attached to *Scirpus spp.* even though the dominant vegetation was *Calamagrostis spp.* We observed no differences between egg mass attachment sites of clear and white egg masses.

Table 9. Maximum number of spotted salamanders egg masses detected at selected breeding ponds at Cape Cod NS in 2001 compared to past surveys (R. Cook, pers. comm.). Shown are the mean number of masses detected during past surveys as well as the maximum number found at each pond for a single year prior to 2001.

	2001 surveys		Past surveys	
Breeding Pond	**Date**	**Egg masses**	**Mean number of egg masses**	**Max number of egg masses**
E1	30 Apr	11	19.8	49
E3	1 May	48	11.25	28
E4	1 May	503	53.25	180
E5	11 Apr	174	32	41
E6	23 Apr	168	106.6	221
E7	23 Apr	92	25	34
E9	22 Apr	469	7.25	12
E10	22 Apr	333	n/a	n/a
E11 (east)	11 Apr	0	n/a	n/a
E11 (west)	11 Apr	101	21.67	33
E12	23 Apr	0	n/a	n/a
P4	22 Apr	0	n/a	n/a
P5	22 Apr	0	n/a	n/a
P9	15 Apr	0	n/a	n/a
P10	22 Apr	0	n/a	n/a
P15	22 Apr	0	n/a	n/a
P16	22 Apr	0	n/a	n/a
T14	24 Apr	3	9	26
T15	24 Apr	42	66.29	91
W7	5 Apr	488	173.83	505
W16	14 Apr	2	1	2
W17	14 Apr	0	n/a	n/a
W18	24 Apr	79	52.25	83

Table 10. Variation in the number of spotted salamander egg masses detected among surveys at ponds at Cape Cod NS during 2001.

	Survey number				
Site	**1**	**2**	**3**	**4**	**5**
E1	6	11			
E3	4	24	48		
E4	450	503			
E9	167	373	469	445	
E10	15	211	333	229	
W7	210	488	444	218	
W18	0	12	79		
T14	0	0	0	3	2
T15	5	6	9	42	17

Table 11. Spotted salamander oviposition attachment substrates and number of clear and milky white egg masses at 9 ponds on Cape Cod NS during 2001 field season.

Pond	**Date**	**Substrate**	**No. Clear**	**No. Milky white**	**No. of Masses**
E1	30 Apr	Bottom	11	0	11
E10	22 Apr	*Sparganium spp.*	32	1	33
E10	23 Apr	*Scirpus spp.*	263	4	267
E10	24 Apr	*Calamagrostis spp.*	34	0	34
E3	1 May	*Decodon verticillatus*	31	4	35
E3	1 May	*Sparganium spp.*	13	0	13
E4	1 May	*Dulichium arundinaceum*	497	6	503
E9	22 Apr	*Juncus spp*	464	5	469
W7	5 Apr	*Decodon verticillatus*	374	114	488
W18	24 Apr	Woody vegetation	75	4	79
T14	2 May	Woody vegetation	3	0	3
T15	24 Apr	*Smilax spp.*	41	1	42

Nocturnal Road Surveys

Road surveys on rainy nights proved to be an effective means for locating *Scaphiopus holbrooki, Bufo fowleri*, and for documenting initial movements of spotted salamander and four-toed salamanders (*Hemidactylium scutatum*). Road surveys were somewhat labor intensive, taking from 52-260 min (mean = 207.3 min, SD = 154.3) for two-to four observers to survey roadways from Eastham to Provincetown (Table 12). Earlier in the season (May and June) road surveys were easily completed. However, traffic becomes dangerous later in the season, due to high traffic volume, making road surveys difficult to complete and potentially hazardous for surveyors.

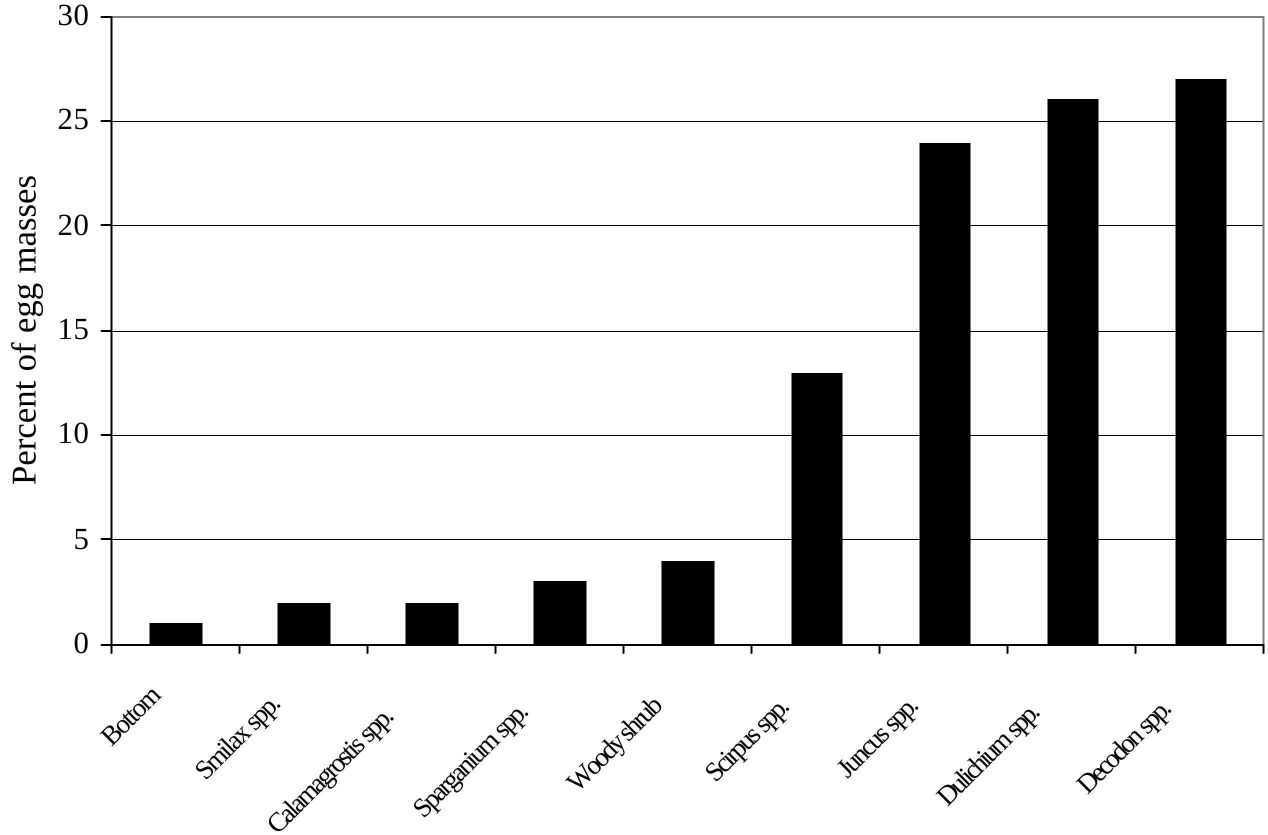


Figure 7. Oviposition microhabitat site selection by spotted salamanders at Cape Cod NS in 2001.

Table 12. Effort expended (total person hours) and total number of specimens located during nocturnal road surveys at Cape Cod NS during 2001.

Date	Total Minutes	Labor Hours	Total No. Located
21 Mar	120	4.0	13
22 Mar	150	5.0	5
6 Apr	85	2.8	9
2 Jun	256	8.5	22
12 Jun	292	4.9	102
17 Jun	496	33.1	243
1 Jul	52	1.7	23

A total of 755 individuals were documented incidentally and on road surveys. The most common species encountered was Fowler's toad, which we found from Eastham to Provincetown (Table 13). We detected 169 Fowler's toads during road surveys. This species was seen every survey from mid-June to late August, although we did not record all observations of this species. Eastern spadefoot were also commonly found on the Cape, with 174 individuals detected during systematic road surveys and 17 individuals incidentally. Observations of spotted salamander (8), four-toed salamander (2), spring peeper (27), American bullfrog (7), green frog (10), red-spotted newt (4) and red backed salamander (5) along roads were also documented on road surveys. We also have a few incidental observations of Fowler's and eastern spadefoots.

Spadefoot toads were found from Eastham (south of Marconi) to Provincetown (Provincelands Road south of Herring Cove) (Table 13) and Fowler's toads showed similar distribution, but were located farther south in Eastham and in greater numbers (Figure 8). Spadefoot toads were surprisingly abundant on the three nights surveyed (12 June, 17 June and 1 July 2001), with 153 individuals (38 other located incidentally) located (46.1 % were found dead on the road). The largest individual was a female (SVL 7.8 cm, 33.9g) and overall (both sexes) mean mass was 12.3 g (SD = 5.73), and mean SVL was 5.01 cm (SD = 0.767). There was a significant correlation between SVL and body mass with $R^2 = 0.804$ (n = 45; Fig. 9). Surprisingly, only six individuals had nuptial pads, all others we classified as either female or juvenile (depending on size).

Three eastern spadefoot breeding areas were identified, two of which had only nocturnal vocalizations detected (behind NPS housing Crapser 5, Truro), and Route 6 at Truro Central Village, see Appendix III, IV, and V), but no larvae or metamorphs were found at these calling areas. One site, two puddles on Hatches Harbor Dike Road, contained *Scaphiopus holbrooki* larvae which metamorphosed and dispersed from the ponds in late June (23 June being the earliest dispersal date) and early July (roughly two weeks after initial oviposition). At the time of measurement (19 June) the two breeding ponds were 9.5 m x 3.9 m and 15 cm deep, and 12.1 m x 5.1 m and 24 cm deep. Larvae were seen in the ponds feeding upside down at the breeding pond surface. All individuals were located at night after periods of rain, though they were seemingly more abundant after heavy rains in June. Eastern spadefoots were heard vocalizing diurnally at Duck Harbor in Wellfleet (Bay Side) on 17 June, and metamorphs were active diurnally in the same area as well. We detected eastern spadefoots into late August, with the last toad found on 20 August 2001.

We documented changes in the spatial distribution of amphibians at Cape Cod NS, with several new town records during the 2001 field season (Table 13). Previously, red-spotted newts were only known from Eastham (Table 1), however we observed two adults in Provincetown at sites P4 and P16 during calling surveys on the night of 22 May 2001. This was significant because newts may occur throughout the four towns of the Seashore. They may have not been previously discovered anywhere other than Eastham because of their primarily aquatic adult lifestyle in large, permanent ponds. Further investigation into the distribution and abundance of this species should be conducted,

Table 13. Town records of all species of amphibians on the Cape Cod National Seashore based on our survey results, (*) indicates a new record in that town.

Species	Eastham	Wellfleet	Truro	Provincetown
Spotted salamander	X	X	X	
Red-spotted newt	X			X*
Red-backed salamander	X	X	X	X
Four-toed salamander			X*	
Eastern spadefoot toad	X	X	X	X
Fowler's toad	X	X	X	X
Spring peeper	X	X	X	X
American bullfrog		X	X*	X*
Green frog	X	X	X	X
Wood frog	X	X*		
Pickerel frog		X	X	

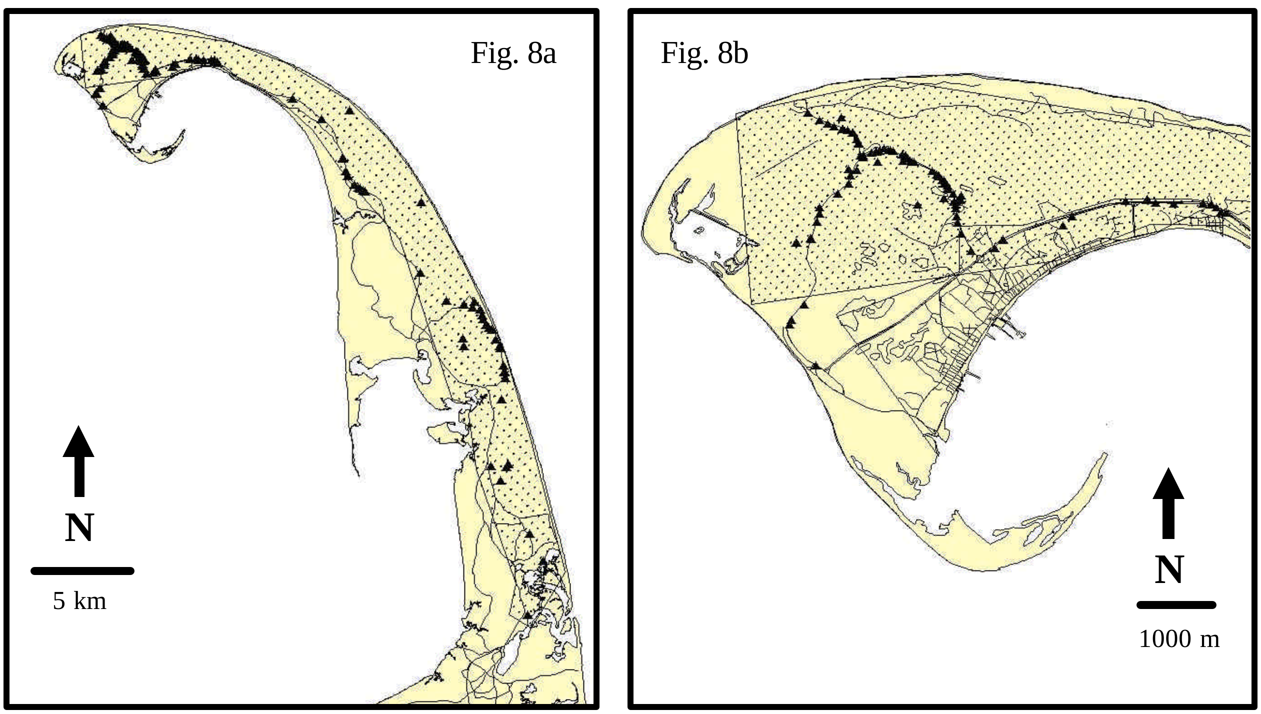


Figure 8a & b. Location of eastern spadefoot toads (triangles) and general distribution of Fowler's toads (stipled area) detected during nocturnal road surveys in 2001 on Lower Cape Cod (Fig. 8a) and near Provincetown (Fig. 8b), within Cape Cod NS. Exact UTM coordinates where eastern spadefoot and Fowler's toads were found on roads are given in Appendix VI.

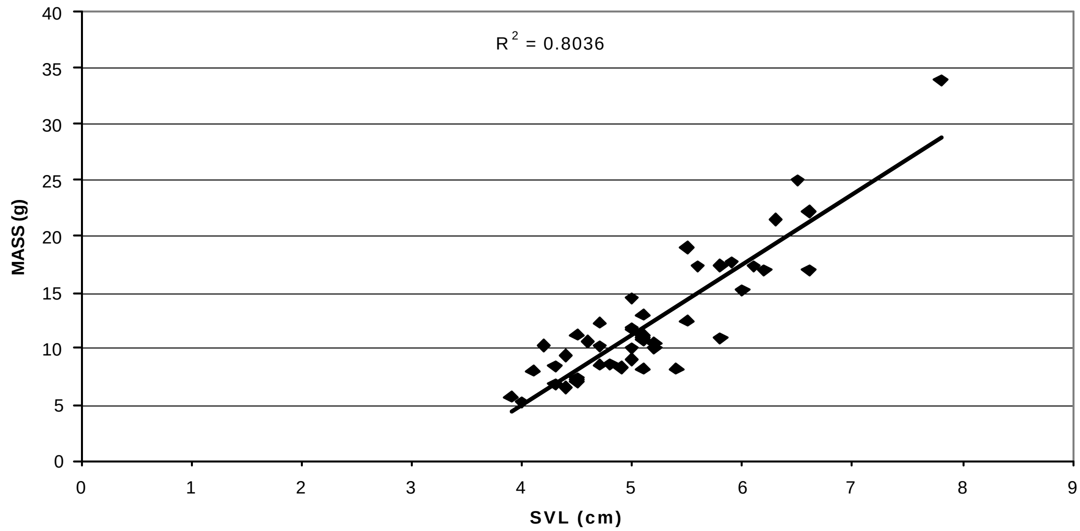


Figure 9. Relationship between snout-vent length and mass for eastern spadefoot toads (*Scaphiopus holbrooki)* collected on Cape Cod NS during 2001 fieldwork.

which could be done with minnow trapping. American bullfrogs previously were found only in Wellfleet and Truro, however one was located calling early in the morning at Lily Pond South during 2001. This also may be evidence of a possible range expansion of this species on the Seashore. Third, wood frogs were only known to occur in Eastham on the Seashore prior to this study. On the night of 6 April 2001 an adult wood frog was seen in a pool of water in the middle of the White Cedar Swamp (site W17), Wellfleet. The individual was not heard calling, however, and we did not detect wood frog egg masses during extensive surveys.

Adult Spotted Salamander Trapping

Minnow trapping (Gee's Minnow Traps, model G-40, 0.63cm mesh size, 43cm long by 22cm wide) for adult spotted salamanders is an effective means of sampling and monitoring spotted salamander populations. Trapping took from 120 and 240 minutes (four and eight person-hours) to check traps, take morphometric measurements, and cohort-toeclipped individuals (mean = 64 min, SD = 73.7). Pattern mapping increased the time required to sample spotted salamander populations with traps. It required roughly 360-min (12 person-hours) to check 22 traps, pattern map 24 individuals, cohort mark and record SVL, TL and mass. The time required to pattern map such a small number of individuals made this technique impractical for recognition of many individuals for long term monitoring. However, it may be more expedient to simply count the number of spots on the head, torso, and tail, without actually mapping the spot pattern, which has been shown to identify up to 97% of individuals and is much less time consuming (Loafman 1991).

Site W7 was the largest pond (2107 m^2) we sampled for spotted salamanders. Based on our population estimates using the Schnabel-Schumacher population estimate, this pond supported the largest population of spotted salamander of all sampled ponds. Six consecutive days of trapping at site E9, the second largest sampled pond (1357.9m^2) produced the second largest *A. maculatum* population estimate and six consecutive days of trapping at T15 the smallest pond sampled, (116 m^2) yielded the smallest population estimate (Table 14).

There were no obvious morphological anomalies of male and female spotted salamanders we trapped. As would be expected (Klemens 1993), at all three sites gravid females were larger than both non-gravid females and males, and non-gravid female were generally larger than males (Table 15).

Table 14. Variation in population size estimates as a function of number of trapping days using Schnabel-Schumacher models for adult spotted salamanders at three ponds at Cape Cod NS.

Pond	Number of trapping days	Mean population size	95% Confidence Interval Lower estimate	95% Confidence Interval Upper Estimate
E9	5	997.3	498.5	n/a
E9	6	805.5	561.5	1424.4
T15	6	100.2	80.7	132.2
W7	3	983.7	318.2	n/a
W7	4	1075.4	748.9	1906.4
W7	5	1238.3	901.8	1975.5
W7	6	1265.4	936.4	1951.0

Table 15. Mean mass (g ± SD), snout-vent length (SVL) (cm ± SD), and total length (TL) (cm ± SD), of spotted salamanders at three ponds (E9, W7 and T15). Parenthesis show SD and sample sizes.

Site	n	Mass (g)	SVL (cm ± SD)	TL (cm ± SD)
E9				
Gravid females	7	20.5 (3.8)	8.6 (0.5)	17.2 (0.9)
Non-gravid females	1	17.4 (n/a)	8.1 (n/a)	16.6 (n/a)
males	33	15.0 (0.0)	7.4 (0.4)	15.2 (0.9)
T15				
Gravid females	20	20.8 (2.9)	8.5 (0.4)	16.6 (0.7)
Non-gravid females	3	14.5 (1.7)	7.6 (0.1)	12.4 (3.7)
males	33	13.6 (2.0)	7.5 (0.4)	15.2 (1.6)
W7				
Gravid females	46	24.1 (3.4)	8.5 (0.7)	16.3 (2.0)
Non-gravid females	26	17.2 (2.8)	7.9 (0.7)	15.5 (1.8)
males	101	13.3 (2.0)	7.2 (0.5)	14.6 (1.3)

Transect Surveys in Deep Kettle Ponds

Each 100-m transect took between 3-6 minutes to complete. During a 6-minute transect walk at Duck Pond for instance, we detected 380 bullfrog tadpoles, and observed some gross malformations of larvae and abnormal larval locomotion. The mean number of tadpoles observed as well as the mean amount of time per transect varied greatly both within and among sites (Table 16)

Table 16. Mean number of bullfrog larvae detected per transect and mean seconds per transect spent surveying each pond.

			No. of larvae		Amount of time (sec)	
Pond	**No. transects**	**Date**	**Mean**	**SD**	**Mean**	**SD**
Duck	6	10 June	205.33	153.66	174	26.8
Gull	3	20 June	20.17	29.68	28.68	25.11
Kinnacum	3	20 June	12.67	17.01	17.01	68.28

Amphibian Habitat Utilization Patterns

Spring peepers were the most ubiquitous anuran on the Lower Cape, as they were located in every wetland category (Table 17). Green frogs were similar to the spring peeper in habitat utilization and were located in every wetland category, however they were not as commonly found in dune slack ponds as peepers, and breeding was generally restricted to waters with semi-permanently and permanently flooded water regimes (Table 18). Pickerel frogs were absent from inter-dunal ponds and vernal ponds, and wood frogs were only present in seasonally flooded vernal ponds. Pickerel frog breeding generally occurred in permanently flooded wetlands. Fowler’s toads were most commonly located in inter-dunal ponds and dune slack ponds, but were present in all wetland habitat categories. Bullfrogs were most commonly encountered in deep kettle ponds and riparian marshes, were only absent from inter-dunal ponds, and breeding only took place in ponds that had permanently flooded hydroperiods.

Table 17. Percent of different wetland types were different species of amphibians were detected using all methods of detection at Cape Cod National Seashore during 2001 field season.

	Habitat type								
Species*	**IDP**	**DSP**	**DKP**	**RM**	**SKP**	**AWCS**	**RMS**	**SS**	**VP**
BUFR	0.00	25.00	100.00	100.00	100.00	0.00	0.00	50.00	18.18
FOTO	100.00	100.00	80.00	25.00	0.00	0.00	0.00	50.00	9.09
EASP	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.18
FTSA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GRFR	100.00	25.00	80.00	75.00	100.00	0.00	100.00	100.00	54.55
PIFR	0.00	25.00	40.00	75.00	0.00	0.00	0.00	0.00	50.00
RSNE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	36.36
SPPE	100.00	100.00	100.00	75.00	100.00	100.00	100.00	100.00	90.91
SPSA	0.00	0.00	20.00	0.00	100.00	0.00	0.00	0.00	54.55
WOFR	0.00	0.00	0.00	0.00	0.00	100.00	100.00	0.00	18.18
N	3	4	5	4	1	1	1	2	11

BUFR=American bullfrog, EASP=eastern spadefoot, FOTO=Fowler's Toad, FTSA=four-toed salamander, GRFR=green frog, PIFR=pickerel frog, RSNE=red-spotted newt, SPPE=spring peeper, SPSA=spotted salamander, WOFR=wood frog, N=sample size. Habitat type: IDP = Inter-dunal Pond; DSP = Dune Slack Pond; DKP = deep kettle pond; RM =riparian marsh; SKP = shallow kettle pond; AWCS = Swamp (Atlantic White Cedar); RMS=red maple swamp; SS = shrub swamp; VP = vernal pool

Table 18. Relationship between pond hydroperiod and probability of detecting each species of amphibian, based on all methods of detection at Cape Cod NS during 2001 field season. Shown is the proportion of ponds with a particular hydroperiod where a given species was detected. N equals number of ponds in each hydroperiod category. * See Table 17 for species codes.

	Hydroperiod			
Species*	**Permanently flooded (always with water**	**Seasonally flooded (dry before August)**	**Seasonally flooded (dry Aug-Nov)**	**Semipermanently flooded (dries some years)**
BUFR	71.43	10.00	20.00	66.67
EASP	0.00	10.00	20.00	0.00
FOTO	57.14	20.00	60.00	33.33
FTSA	0.00	0.00	0.00	0.00
GRFR	92.86	30.00	60.00	66.67
PIFR	42.86	0.00	20.00	0.00
RSNE	0.00	20.00	20.00	33.33
SPPE	100.00	90.00	100.00	66.67
SPSA	14.29	30.00	20.00	66.67
WOFR	0.00	20.00	20.00	33.33
N	14	10	5	3

DISCUSSION

Manual Anuran Call Surveys

Calling anuran surveys are an effective way to monitor anuran species at Cape Cod NS and should be one of the primary survey techniques adopted for a long-term monitoring program at the seashore. We evaluated three sources of variation (seasonal, diel, and environmental) in male advertisement calls that should be considered when developing a long-term anuran monitoring program. Assessing seasonal variation in call behavior is critical because each species has a well-defined time period when detection probabilities are high (Wright and Wright 1949, Bishop *et al*. 1997, Corn *et al*. 2000, Crouch and Paton 2002). Thus, periods with high detection probabilities, which we term *survey windows*, need to be identified locally throughout North America because there can be considerable latitudinal variation in timing of male advertisement calls. This is particularly true for species that breed early in the spring, as there tends to be more latitudinal variation in chorus initiation dates for early breeding species such as *R. sylvatica* and *P. crucifer* (Crouch and Paton 2002). Because there is a higher probability of stochastic environmental events constraining male calling activity for early breeding species, there will be more annual fluctuations in survey windows for species that breed in March (*e.g.*, *R. sylvatica*, Mossman *et al.* 1998, Crouch and Paton 2000) compared to species that breed in June (*e.g.*, *R. clamitans*). This is why current NAAMP protocols allow regional coordinators the flexibility to vary the first survey window for wood frogs based on weather conditions (S. Jackson, NAAMP regional coordinator, Univ. of Massachusetts, Amherst, pers. comm.).

During our study, species-specific detection probabilities ranged from 0.47 to 0.81 within survey windows. However, detection probabilities dropped rapidly to 0 outside of survey windows, thus identifying survey windows is the first critical step before embarking on a monitoring program. We documented 3 14-day survey windows when call surveys should be conducted at Cape Cod to maximize detection probabilities; 21 March to 3 April for *R. sylvatica*, 20 April to 3 May for *R. palustris* and *P. crucifer*, and 30 May to 13 June for *B. fowleri*, *R. clamitans*, and *R. catesbeiana*. This time schedule would allow for 14-day intervals between survey windows as suggested by NAAMP (2002) guidelines. In contrast, the Massachusetts NAAMP program (S. Jackson, pers. comm.) and Crouch and Paton (2002) suggest 4 surveys windows are needed to monitor all species in the region. This is due in part to the fact that Cape Cod has a relatively depauperate anuran fauna compared to the rest of the region, thus 4 survey windows are necessary in other parts of southern New England. Detection probabilities within survey windows we documented were similar to estimates from Rhode Island (Crouch and Paton 2002); *R. palustris* had a relatively low detection probability, *R. sylvatica* and *R. catesbeiana* had moderate detection probabilities , while *R. clamitans* and *P. crucifer* had relatively high detection probabilities. Thus it appears that detection probabilities for a given species are relatively consistent within a region, which makes it simpler to design a monitoring program.

In contrast to Mohr and Dorcas (1999) and Bridges and Dorcas (2000), we found that current NAAMP (2002) time guidelines suggesting that call surveys should be conducted from 30 min after sunset to 0100 hr were generally appropriate for our study area. One exception was *R. sylvatica*, which we detected calling throughout the afternoon and early evening, thus call surveys for this species in coastal New England should be conducted earlier than current NAAMP guidelines (see also Crouch and Paton 2000, 2002). At Cape Cod, we documented male advertisement calls of *R. clamitans* and *R. catesbeiana* peaked near midnight, whereas both species often did not reach peak calling intensity until 0400 hr in South Carolina (Mohr and Dorcas 1999). This shows that there is regional variation in diel calling activity patterns for the same species. We concur with Bridges and Dorcas (2000) that diel variation needs to be quantified locally to ensure that all species are being monitored when detection probabilities are high during survey windows.

We were unable to detect all anuran species at Cape Cod NS with call surveys. Eastern spadefoot toads were never heard during manual call surveys or on tapes collected by ARS. This was somewhat surprising as the species is common in the Provincetown area; we observed almost 200 individuals during nighttime road surveys. Therefore, call surveys are not appropriate for all anurans that breed in coastal New England. Corn *et al.* (2000) provide an excellent summary of when manual call surveys and ARSs may be appropriate if one is designing a monitoring program. In addition, Heyer *et al.* (1994) summarized a variety of survey techniques available to assess amphibian populations.

During our study, surface water temperature was more likely to affect calling rates than air temperature. This makes biological sense, as males of most species we monitored generally immersed themselves in water while calling. One exception was *B. fowleri*, which tended to stand upright in shallow water when calling. This may explain why *B. fowleri* call activity was more affected by air temperature than surface water temperature. Our study also suggests that when conducting call surveys, biologists should consider constraining survey occasions to nights when surface water temperatures exceed minimum threshold values (*e.g.*, ≥10 °C) in coastal New England, rather than using air temperature guidelines. However, this is more difficult to do than monitoring air temperatures, thus using water surface temperature constraints may not always be practical. We did find that anurans at Cape Cod rarely called when air temperatures were <7 °C, which suggests that the current NAAMP air temperature threshold (5.6 °C) may be too low.

Using call surveys to monitor changes in occupancy rates of wetlands by anurans will provide useful information on long-term amphibian population trends. Reports of anthropogenic declines in amphibian populations throughout the world are a major concern for biologists (Mattoon 2001). However, much of the evidence purporting to show declines is anecdotal, as few long-term amphibian monitoring programs have been initiated (Pechmann *et al.* 1991). Therefore, there is a pressing need to initiate long-term monitoring programs to assess population trends.

Call surveys are one technique that biologists could use to monitor anuran populations. However, our research shows that biologists need to carefully consider regional variation in conducting surveys to maximize detection probabilities. Call surveys are now used nationwide, thus data collected at the Seashore could be used to help regional and national population trends. Call surveys, initially based on the breeding bird survey (BBS), are an effective means to assess anuran population trends (Crouch and Paton 2002), although calling surveys are more effective for common species. While call surveys do not allow biologists to obtain precise estimates of the number of individuals present at a site, they are an effective way to track breeding population trends. Call surveys require relatively little labor compared to many other survey techniques. Particularly for common species at the Seashore (*e.g.*, spring peeper, green frog), relatively few surveys are required to monitor population trends (refer to Table 5). Another advantage of call surveys is that observers can be trained quickly to identify all anuran calls at the Seashore. There are only 6 species that are typically encountered and their calls are readily distinguishable. Tapes are readily available of all anurans that call at the Seashore. Other advantages of this technique are that it has minimal impact on the environment, requires few materials, and takes a relatively short amount of time to collect a considerable data. Although the analyses presented here and work by Crouch and Paton (2002) suggest that only presence/absence data may be required to monitor long-term anuran population trends, we suggest that observers continue to record categorical calling index data during surveys at the Seashore. The current NAAMP protocol still uses calling indices, thus data collected at the Seashore should be comparable to the national standard.

Automated Recording Systems (ARS)

Based on our experience with ARS, they are to not a useful technique to monitor long-term population trends. They are extremely time intensive, they are also fairly unreliable in marginal weather conditions (cold weather, high winds, and high humidity). Also they are somewhat expensive to build/purchase (roughly $500 per unit) and they can be subject to vandalism and theft in areas where people may frequent (although this never happened to us, except that a mouse or other small animal chewed through wires and made one unit nonfunctional for 1 month). ARSs are useful for quantifying aspects of amphibian ecology such as diel and seasonal variation calling chronology, as well as for potential documentation of the presence of a particular anuran species at a site. Other drawbacks to ARSs are that they are labor intensive because they require visits to the site every 48 hours, and another 100 min of transcribing for each 90 tape (which represents 4 days of ARS recordings). Because they are so labor intensive and relatively expensive, few sites could easily be monitored with ARS, therefore they would not be useful for a long-term monitoring program at the Seashore.

Tadpole Dyeing

While Jung *et al.* (2002) found tadpole dyeing of green frogs to be an effective technique to estimate larval populations at a site we were unable to repeat their successes. Even when using concentrations greater than originally proposed that we usually could not discern dyed animals three hours after being placed in dye. One reason could be that the tails of green frog tadpoles are heavily mottled and fairly dark throughout and therefore do not contrast significantly with the dye. Species that have less pigmentation on the tails of tadpoles (*e.g.* Fowler's toad and wood frog) might allow more contrast more with the dye and would probably be more effective subjects for this technique, but other factors must first be investigated. We found there was some mortality at higher dye concentrations (Table 6), and the survival threshold of dye concentration varies among species. Also, as the method has been proposed, the dyed individuals are to be returned to the site after exposure to the dye and then the pond is to be dipnetted 3 hours later. In order for this technique to be a valid population estimator, it assumes that the behavior of the dyed individuals will not be altered in the process, which has not be tested in the field. Finally, it is our feeling that this technique would be very labor and time intensive. It requires considerable time spent in the pond initially dip-netting larvae, then dying them, releasing them, capturing them a second time, and then carefully inspecting each individual to see if it is dyed or not. Because tadpoles are in ponds at different times of the year (Paton and Crouch 2002), multiple visits would have to be conducted annually to sample the entire community. It would be much more cost effective to use calling surveys to assess the relative abundance to breeding adults at ponds, rather than dyeing which will also provide an index to breeding population size. No one has effectively used tadpole densities to track adult population sizes to our knowledge.

Dip-netting

This technique could be an effective means of tracking larval population trends, however this technique is also very labor intensive and requires observers with greater training than calling surveys. Identifying tadpoles and salamander larvae can be difficult and requires a microscope and laboratory time. Larvae of some species of amphibians are difficult to discern from one another, but an experienced observer should be able to differentiate between species. One clear advantage of this technique is that all pond-breeding amphibian larvae can be sampled, whereas call surveys only monitor anurans. Thus, dip net sweeps are one of the few techniques available to monitor caudates (salamanders). However, sites would have to be surveyed several times annually, due to interspecific differences in larval metamorphosis. Another advantage of this technique is that the invertebrate structure of each dip net site can be sampled concurrently. Invertebrate community structure can negatively impact on amphibian populations, and therefore may allow observers to gain a clearer understanding of the factors that influence amphibian populations. Again, sampling aquatic invertebrates is beyond the scope of the long-term monitoring goals and requires considerable expertise. Another disadvantage to dip-netting is that it is fairly disruptive to the breeding habitat as well as to pond organisms (amphibians and other taxa alike) when biologists scrape through pond bottom to capture larvae. However, disturbance could be minimized. We believe that dip-netting is too labor intensive, too imprecise, and requires too much laboratory time to identify larvae to be a practical survey technique for a monitoring program at Cape Cod.

Egg Mass Counts

Egg mass counts are an extremely effective technique to monitor breeding populations of wood frogs and spotted salamanders. Paton and Crouch (2000) found that there is a 1:1 relationship between the number of wood frog egg masses found in a pond to the number of females breeding at the site. While available evidence suggests there is not as strong a relationship with egg masses and the number of breeding female spotted salamanders at a site, it is still a useful index of the breeding effort of this species. Egg mass counts are easily comparable between years and require minimal labor. Ponds only have to be visited once or twice during the season (Egan 2001), and the dates in which these surveys need to be conducted are fairly predictable. Also observers can be trained in a short period of time to perform these censuses and because these surveys take a relatively short period of time to conduct, a large number of sites could be sampled each season. Therefore, we suggest this is another survey technique that the Cape Cod NS adopt to monitor long-term population trends on pond-breeding amphibians at the Seashore.

Nocturnal Road Surveys

Road surveys have limited usefulness for a monitoring program, however they are an effective means to identify potential breeding ponds of all species of pond-breeding amphibians, especially in a Park such as Cape Cod NS that is fragmented by so many roads. Nocturnal road surveys might be the only effective technique to monitor eastern spadefoot, particularly near Provincetown. Determining when to conduct surveys is relatively predictable, as eastern spadefoots are usually active from May-August during nights of heavy rainfall (Klemens 1993). During nocturnal road surveys we documented 153 individuals of this species, while we only know of three other occasions during this field season in which this species was documented calling on the Seashore. The low number of encounters based on calling surveys and incidental calling encounters alone may lead to false assumptions about the size of the populations here, and road surveys would help in that respect. If Cape Cod NS would standardized the road segments to be surveyed on rainy nights from May-August, it would be possible to develop a standardized survey method that could be repeatable among years.

Considering the fact that our results suggest that Cape Cod NS represents the northernmost large concentrations of eastern spadefoots in North America, this population should be monitored in the future. The Park might even consider closing certain segments of road on rainy nights because eastern spadefoot are a state listed threatened species and our data show that large numbers of eastern spadefoot are being killed during these storm events by vehicles. In fact, during a survey in June 2001, one of the only vehicles on the road during a storm was a Park ranger, who probably was responsible for driving over a significant number of eastern spadefoots.

One of the main problems with road surveys is that they are a relatively dangerous technique for surveyors because this technique involves driving on roads at night, stopping abruptly each time an amphibian is encountered on the roadway, and getting out of the vehicle to take the individual off the road. It is imperative that observers wear extensive reflective gear when conducting surveys, flashers are turned on the vehicle, and NPS might even consider adding flashing yellow lights to the tops of survey vehicles.

Adult Spotted Salamander Trapping

Trapping adult spotted salamanders is probably not a very effective monitoring technique to use for a variety of reasons. First, it is difficult to standardize the dates in which the traps should be run. Usually spotted salamanders in this region tend to migrate to breeding sites in early to mid-March during warm, rainy nights (Paton and Crouch 2002, Egan 2001, B. Timm, pers. obs.). However, it is often difficult to predict when animals are immigrating to ponds. In many cases, as was the situation during the 2001 field season on the Seashore, there were several nights in which spotted salamander adults were found migrating to breeding sites. Also the activity of the salamanders while at their breeding ponds is variable between nights at the same site (*e.g.* at site W7 the number of individuals trapped during the night of 24 March and early morning of 25 March totaled 302 while the same traps were checked one day later and only 18 individuals were captured) also the activity dates are variable between sites, presumably this is due to factors such as fluctuations in air and water temperature. One solution would be to run traps every night for a period of a month or so at several sites. However, trapping is very time consuming (especially during days of high capture rates), it can be destructive to breeding habitat (a lot of silt is kicked up while collecting traps, and egg masses may inadvertently be walked upon, vegetation is often disturbed), it may have a significant direct impact on the animals (it may inhibit their reproduction by being trapped during nights of potential breeding, individuals may be injured while in the traps either when they enter the trap or by invertebrates concurrently caught in the trap, and in several cases we found females who deposited their egg masses while inside of the traps), and may end up killing or injuring other pond organisms (*e.g.* tadpoles and invertebrates). Marking animals is very time consuming and expensive. We were not successful with monitoring individuals using spot patterns, however we did not attempt to simply count spot patterns as Loafman (1991) suggested. This may be an alternative technique if NPS decides it wants to trap salamanders to monitor long-term population trends.

Larval Spotted Salamander Trapping

We found little evidence to support using minnow traps to monitor larval spotted salamander populations. The same problems exist for this method of trapping as were listed dip-netting. Another problem with this method is that the minnow traps used had entrances that were large enough for nearly metamorphosed larvae to escape. Even if modified traps were used to avoid this, we believe this technique to too unreliable to recommend this as a monitor technique for NPS.

Transect Surveys in Ponds

Transect surveys would be an effective technique in assessing the larval populations of green frogs and American bullfrogs in large deep kettle ponds and inter-dunal ponds of Cape Cod NS. This might be useful program to initiate because there have been some die-offs of anurans at larger deep kettle ponds (R. Cook, NPS, pers. comm.). If Cape Cod NS hopes to track this phenomena, initiating walking transects in ponds would require minimal labor and time, and no materials. Dip-netting would be an alternative method of sampling for these and other species in smaller ponds such as vernal pools. However, in large deep kettle ponds, dip-netting would be a poor technique to monitor amphibian productivity because there would be relatively low capture rates. By using a fairly randomized scheme of 100-m transect walks is these larger, clear ponds, observers would be able to fairly accurately monitor these larval populations between years. This technique is also low-impact on the environment sampled as well as on the organisms directly.

PART TWO
The Amphibian Monitoring Protocol

Based on fieldwork conducted in 2001, two techniques will be used to monitor the long-term population dynamics of amphibians at Cape Cod National Seashore (see Part I). These techniques are Anuran Call Surveys and Egg Mass Counts. Anuran call surveys were selected because they are a relatively inexpensive way to survey amphibian occurrence and relative abundance at a large number of wetlands, and available evidence suggests that calling surveys can be a powerful tool to track changes in community composition and population size over time (Shirose *et al.* 1997, Crouch and Paton 2002, Paton *et al.* In review). In addition, data gathered at Cape Cod NS can contribute to National Monitoring efforts, such as the North American Amphibian Monitoring Program (NAAMP). Egg mass counts are also an effective and relatively inexpensive way to monitor the occurrence and abundance of breeding populations of selected species that do not vocalize or are difficult to detect vocally, and lay readily observable and identifiable egg masses. Finally, a third method, Nocturnal Road Surveys, may be useful for monitoring spadefoot toads in the Province Lands. Its utility warrants further study.

Anuran Call Surveys

Anuran call surveys will be modeled after the North American Amphibian Monitoring Program (NAAMP) in the use of a nightly "route survey" consisting of 10 sampling sites/route. Given the large number and variety of wetland types at CACO, the park's linear nature, and the known south to north variation in the distribution and abundance of some species, three routes will be surveyed (30 sites total). As described in Part 1, sampling sites were selected based on a stratified random selection of sites within eight types or categories of wetlands present at Cape Cod NS and among the four towns that comprise the park. While any single individual route does not contain all possible wetland types (due to the geographic distribution of wetland types), collectively the 30 sites sample across all wetland types as well as south to north.

Where CACO's monitoring program varies with NAAMP is in the sampling schedule. NAAMP utilizes survey "windows" based on calling phenology of potentially-occurring species, with intervals of no sampling in between. Rather than sample certain sites at certain times for a particular species, surveys at CACO will be conducted weekly at all 30 sites. This will minimize the potential impacts of annual variation in weather as well as long term climate change, and, with this greater amount of survey effort, increase the likelihood of detecting species with lower detection probabilities. Moreover, by sampling this geographically and ecologically diverse group of wetlands across the entire amphibian calling season, long term changes in phenology, species distributions, and community composition should be detectable. However, because surveys will include the NAAMP survey windows, the appropriate subsets can be provided to that program.

Sampling Sites

The three survey routes and 30 sampling sites are shown below (Table 19 and in Figures 10, 11, and 12). Since each site will be sampled repeatedly from the same point, prior to data collection, at least one field crew member will visit each of the sites in daylight, to ensure familiarity with sites and exact sampling points.

Table 19. Anuran call survey routes, site location, wetland type, and UTM coordinates at Cape Cod National Seashore. E: Eastham, W: Wellfleet, T: Truro, P: Provincetown.
* Sites that were not sampled during the 2001 protocol development field season.

Route	Site	Town	Wetland Type	Easting	Northing
1	E15	E	Swamp-red maple	419780	4630402
1	E18	E	Vernal Pool	419354	4632317
1	E9	E	Vernal Pool	420056	4633659
1	E4	E	Vernal Pool	420298	4634044
1	E16	E	Vernal Pool	420925	4634371
1	W18	W	Vernal Pool	418750	4640381
1	W17	W	Swamp-white cedar	418675	4640450
1	W15*	W	Vernal Pool	418064	4641832
1	Kinnacum Pond	W	Deep Kettle Pond	417287	4644916
1	W7	W	Vernal Pool	417431	4644996
2	Grassy Pond	W	Shallow Kettle Pond	416901	4643453
2	Herring Pond	W	Deep Kettle Pond	416167	4646070
2	Black Pond	W	Riparian Marsh	415083	4646131
2	Snow Pond	T	Deep Kettle Pond	414887	4646816
2	T15	T	Vernal Pool	414263	4646623
2	T01*	T	Vernal Pool	412586	4648436
2	Ballston Marsh	T	Riparian Marsh	415412	4650231
2	Pamet Bog	T	Bog	414806	4650581
2	T31*	T	Vernal Pool	408272	4656609
2	T18*	T	Dune Slack Pond	407323	4657397
3	P40*	P	Dune Slack Pond	403874	4658161
3	P20*	P	Interdune Pond	401753	4657811
3	P21*	P	Vernal Pool	401667	4658008
3	Grassy 1*	P	Interdune Pond	401823	4658126
3	P5	P	Dune Slack Pond	401414	4658014
3	Lily Pond 3	P	Interdune Pond	401061	4657857
3	Great Pond 1	P	Interdune Pond	400801	4657799
3	P13	P	Dune Slack Pond	400256	4658421
3	P8	P	Dune Slack Pond	400634	4658918
3	P6	P	Dune Slack Pond	399857	4658332

Figure 10. Anuran calling count, Route 1.

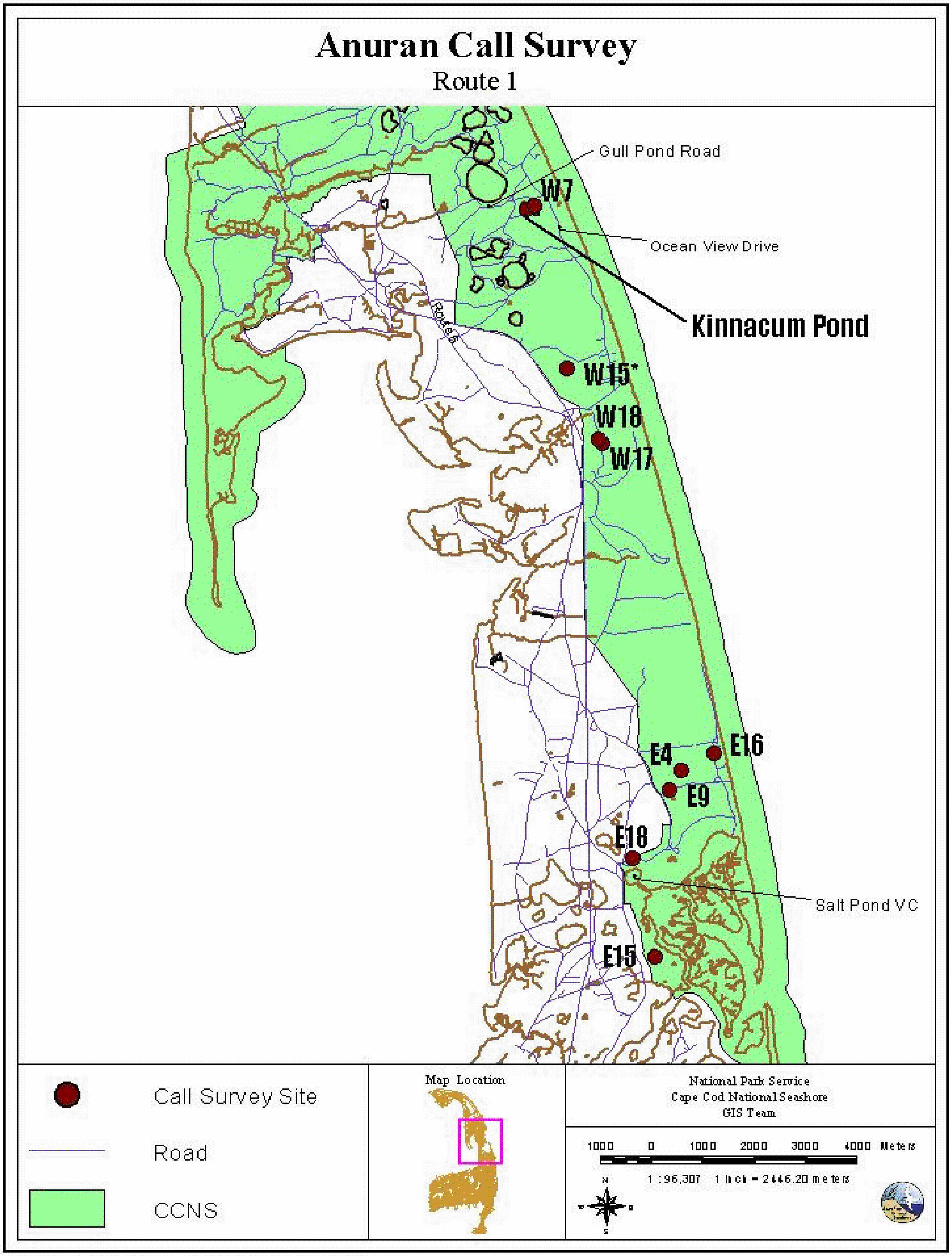


Figure 11. Anuran Call Count, Route 2.

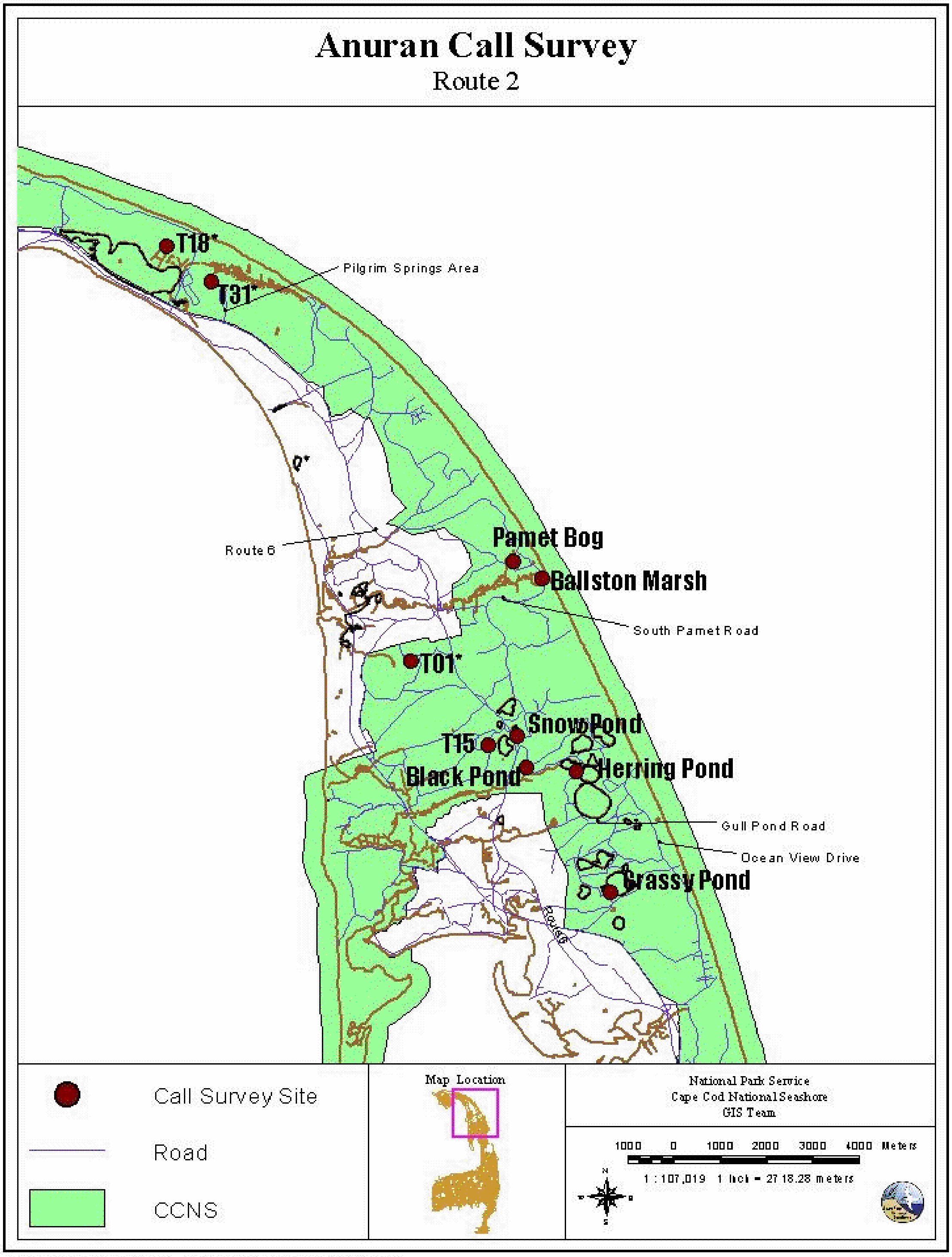
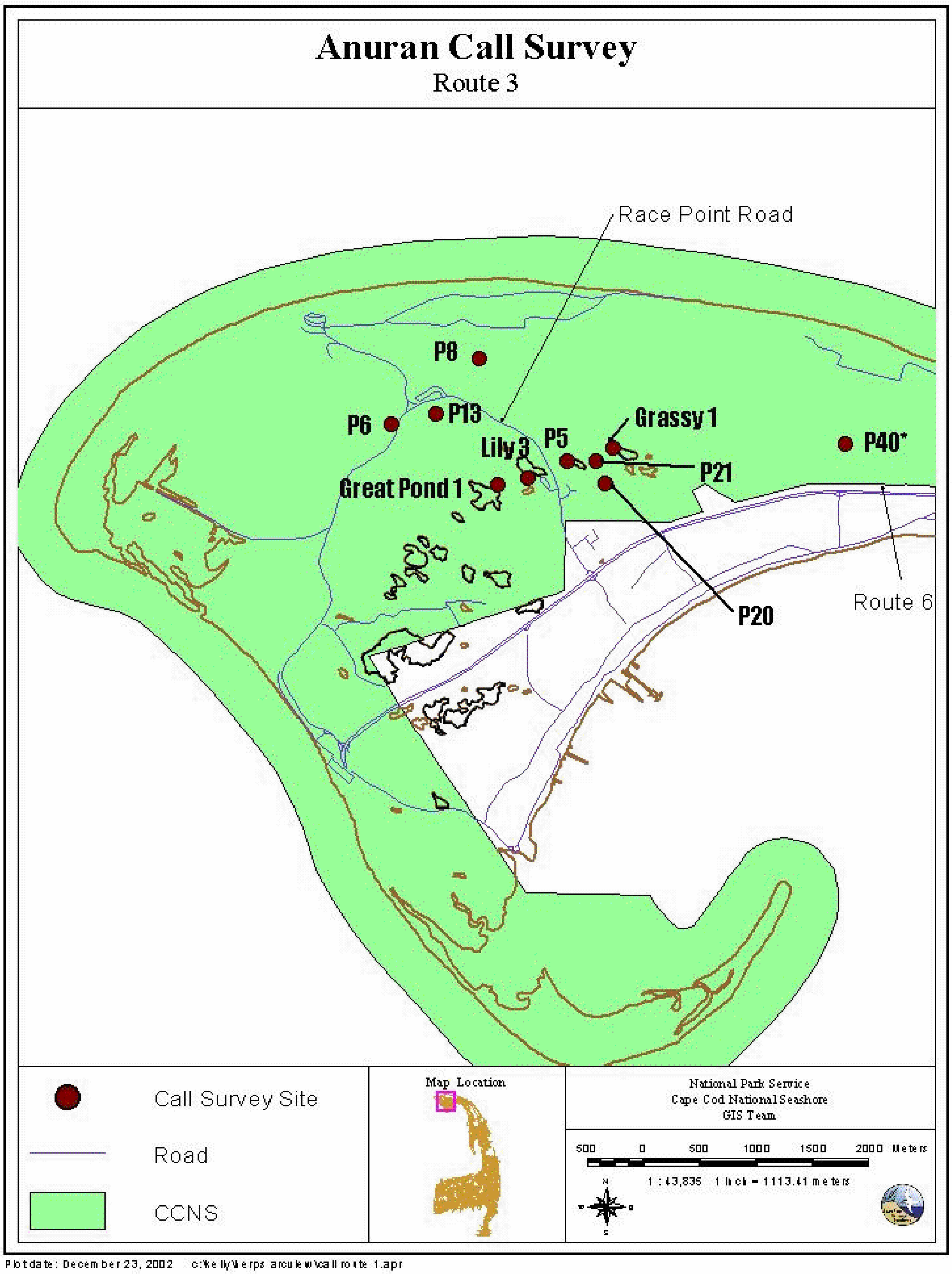


Figure 12. Anuran Calling Count, Route 3.



Sampling Schedule

Surveys will be conducted weekly from ca. 15 April to ca. 15 July, with 12-13 surveys total conducted in a season. Ideally, surveys will be conducted during the traditional Monday-Friday workweek, with the default weekly schedule to be Tuesday, Wednesday, and Thursday night. However, since it is likely that in some years, particularly in mid-April, conditions on a given night or two may be outside those described below, some flexibility is needed. Therefore, in any given week, weather forecasts will be consulted on Monday and the weekly survey schedule set to avoid, as much as possible, sampling on nights where conditions fall outside those described below.

Order of Sampling

To avoid sampling sites within a survey route at the same time each survey, the order of sampling will vary from week to week. While a random order may be theoretically preferable, it is not logistically practical. Instead, an approach that attempts to balance these considerations will be used. For the first week's survey, sites will be sampled from south to north, whereas in the second they will be generally sampled north to south. However, since an exact reversal of order will cause sites in the middle of the order to remain there, when sites within a route are clustered together, the order of sampling within that cluster will also be varied (Table 20).

Time of Sampling

Based on research at Cape Cod NS, calling anuran surveys will be conducted from dusk until midnight, which is when most species are calling with the greatest intensity (Paton *et al.* In review).

Time Required

Each weekly sampling run will require 3 nights, between dusk and midnight, with ca. 4 hours/night anticipated field time.

Sampling Conditions

Because CACO anurans tended to be more sensitive to surface water temperature than air temperature, every effort should be made to conduct surveys when surface water temperatures are at least 50 °F (10°C). In addition, as called for in the NAAMP protocol, surveys should be conducted when wind scale is three or less (a gentle breeze or less) and in the absence of heavy rain. As described above, weekly survey schedules will be modified to survey on nights that, for each given week, most closely meet these conditions.

Environmental Data

Data on air and water temperatures, and wind, sky, and noise will be collected in the course of conducting the call counts. Data on pond pH (and other water quality parameters) will be collected as part of a broader program of water quality monitoring.

Equipment List

During each stop on the calling survey route the observer(s) should bring with them to each stop:

- (1) stopwatch (preferably one with a luminescent display; LCD)
- (1) flashlight and/or headlamp for each observer
- (1) clipboard with the calling survey form attached (Table 21) (on rainy nights use forms copied onto waterproof paper)
- (several) graphite mechanical pencils
- (1) water thermometer
- (1) air thermometer
- (ample) backup batteries for the flashlights/headlamps
- hip waders/rubber boots for observers

Table 20. Weekly sampling order of sites within each call survey route. E: Eastham, W: Wellfleet, T: Truro, P: Provincetown. * Sites that were not sampled during the 2001 protocol development field season.

			Survey Week #												
Route	**Site**	**Town**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	E15	E	1	10	1	10	1	10	1	10	1	10	1	10	1
1	E18	E	2	9	2	9	2	9	2	9	2	9	2	9	2
1	E9	E	3	7	3	7	3	7	3	7	3	7	3	7	3
1	E4	E	4	8	4	8	4	8	4	8	4	8	4	8	4
1	E16	E	5	6	5	6	5	6	5	6	5	6	5	6	5
1	W18	W	6	4	6	4	6	4	6	4	6	4	6	4	6
1	W17	W	7	5	7	5	7	5	7	5	7	5	7	5	7
1	W15*	W	8	3	8	3	8	3	8	3	8	3	8	3	8
1	Kinnacum Pond	W	9	2	9	2	9	2	9	2	9	2	9	2	9
1	W7	W	10	1	10	1	10	1	10	1	10	1	10	1	10
2	Grassy Pond	W	1	4	1	4	1	4	1	4	1	4	1	4	1
2	Herring Pond	W	2	6	2	6	2	6	2	6	2	6	2	6	2
2	Black Pond	W	3	5	3	5	3	5	3	5	3	5	3	5	3
2	Snow Pond	T	4	7	4	7	4	7	4	7	4	7	4	7	4
2	T15	T	5	8	5	8	5	8	5	8	5	8	5	8	5
2	T01*	T	6	9	6	9	6	9	6	9	6	9	6	9	6
2	Ballston Marsh	T	7	10	7	10	7	10	7	10	7	10	7	10	7
2	Pamet Bog	T	8	3	8	3	8	3	8	3	8	3	8	3	8
2	T31*	T	9	2	9	2	9	2	9	2	9	2	9	2	9
2	T18*	T	10	1	10	1	10	1	10	1	10	1	10	1	10
3	P40*	P	1	10	1	10	1	10	1	10	1	10	1	10	1
3	P20*	P	2	4	2	4	2	4	2	4	2	4	2	4	2
3	P21*	P	3	5	3	5	3	5	3	5	3	5	3	5	3
3	Grassy 1*	P	4	6	4	6	4	6	4	6	4	6	4	6	4
3	P5	P	5	7	5	7	5	7	5	7	5	7	5	7	5
3	Lily Pond 3	P	6	8	6	8	6	8	6	8	6	8	6	8	6
3	Great Pond 1	P	7	9	7	9	7	9	7	9	7	9	7	9	7
3	P13	P	8	2	8	2	8	2	8	2	8	2	8	2	8
3	P8	P	9	3	9	3	9	3	9	3	9	3	9	3	9
3	P6	P	10	1	10	1	10	1	10	1	10	1	10	1	10

Protocol

Weekly survey runs will be conducted in accordance with the sampling schedule, weekly sampling order, time of night, and weather conditions described above. To better ensure that the pre-determined sampling order for a given run is followed, it is advisable to fill in the site names on the field data form (Table 21) prior to beginning the field work. At the start of the night's calling survey route the individual will record the following information on the field data form (Table 21): Survey Route #, Run#, Date, Observer(s), #days Since Last Rain. At each individual sampling site the following additional information will be collected as described below, and recorded on the same field data form; Site Name, Start Time, End Time, Air Temp (F^o), Water Temp (F^o), Wind Code (Table 22), and Sky Code (Table 23), # of cars passing during the 5 minute survey period, Noise Code (Table 24), and Calling Index value (Table 25) for every anuran species heard calling during the 5 minute survey period.

In conducting weekly survey runs, the observer(s) will get out of the vehicle and walk, as quietly, and non-disruptively as possible, to the assigned survey point (as marked by rebar) at each site. Then, at this first calling survey stop, and at every subsequent stop, the observer will record the site name and the start time (military) then begin the calling survey (In accordance with NAAMP protocols, there is no initial waiting period, the survey begins immediately). At the same time as the initiation of the survey, the observer will begin recording the air temperature. The observer will record each anuran species heard vocalizing during the five minute survey and record the highest calling index (Table 25) each species attained. In addition, the observer will keep count and record the number of cars that passed by the site during the five minute survey. At the end of the five minutes the observer will record the noise index (Table 24) for the survey, take the reading from the air temperature thermometer, sum the number of cars that passed during the five-minute survey, and then proceed to the water's edge of the site to take the water temperature. To take the water temperature, the observer will use a thermometer and place it in the water so that it is completely submerged, and keep it there for at least 60 seconds. After the water thermometer has been submerged for at least 60 seconds, the observer will remove it from the water and immediately take the reading (degrees Fahrenheit for greater precision) and record it in the designated location on the data sheet. At any time during and/or after the survey the observer will write down any notes that may be of interest (*e.g.* behavioral observations, bright moon, wildlife observations, wind changes etc.) on the bottom of the data sheet. Of particular interest are any anurans observed or heard that were not recorded during the official survey period. Once all of the necessary data has been collected for each site, the observer will return to the vehicle and drive to the next site, repeating this process until all the sites for that evening's run have been surveyed.

Table 21. Field Data Form for Anuran Call Count Surveys.

ANURAN CALL COUNTS - FIELD DATA

Route #			**Event comments:**								
Week#											
Date											
Observers											
# Days Since Last Rain											
	Stop1	**Stop2**	**Stop3**	**Stop4**	**Stop5**	**Stop6**	**Stop7**	**Stop8**	**Stop9**	**Stop10**	**Stop____**
Site Name											
Start Time											
End Time											
Air Temp F^o											
Water Temp F^o											
Wind Code											
Sky Code											
Noise											
#Cars											
Species/Index											
Spadefoot Toad											
Fowler's Toad											
Spring peeper											
Grey Treefrog											
Wood Frog											
Pickerel Frog											
Green Frog											
BullFrog											

Survey comments

Table 22. Beaufort wind scale codes based on NAAMP guidelines.

Beaufort Wind Codes	
0	Calm (<1mph) Smoke rises vertically
1	Light Air (1-3 mph) smoke drifts, weather vane inactive
2	Light Breeze (4-7 mph) leaves rustle, can feel wind on face
3	Gentle Breeze (8-12 mph) leaves and twigs move around, small flags extend
4*	Moderate Breeze (13-18 mph) moves thin branches, raises loose papers * Do not conduct survey at Level 4, unless in Great Plains
5**	Fresh Breeze (19 mph or greater) small trees begin to sway ** Do not conduct survey at Level 5 in ALL REGIONS

Table 23. Sky codes based on NAAMP guidelines.

Sky Codes (note 3 and 6 are not valid code numbers)	
0	Few Clouds
1	Partly cloudy (scattered) or variable sky
2	Cloudy or overcast
4	Fog or smoke
5	Drizzle or light rain (not affecting hearing ability)
7	Snow
8	Showers (is affecting hearing ability). Do not conduct survey

Table 24. Massachusetts noise index based on NAAMP guidelines.

Noise Index	
Massachusetts Noise Index	Definition
0	No appreciable effect (*e.g.* owl calling)
1	Slightly affecting sampling (*e.g.* distant traffic, dog barking, one car passing)
2	Moderately affecting sampling (*e.g.* nearby traffic, 2-5 cars passing)
3	Seriously affecting sampling (*e.g.* continuous traffic nearby, 6-10 cars passing)
4	Profoundly affecting sampling (*e.g.* continuous traffic passing, construction noise)

Table 25. Calling indices used in surveys based on NAAMP guidelines

Amphibian Calling Index	
0	None heard calling
1	Individuals can be counted; there is space between calls
2	Calls of individuals can be distinguished but there is some overlapping of calls
3	Full chorus, calls are constant, continuous and overlapping

Data Entry

For each calling anuran survey route conducted a field data sheet (Table 21) will be filled out, and from that the data will be transcribed into an Access database. Observers will enter the data into the database no later than 24 hours after the completion of the survey in order to minimize data entry errors (observers may realize the next day that fields were inadvertently incorrect, if this is the case the data form should be corrected). All calling surveys from a season will be entered into the same database and should be proofed with the raw data before any analyses are initiated. Data should be saved to the computer's hard drive, and on the Biolab "Y" drive "dataarchives", and on a disk or cd. All raw data sheets will be three-hole punched, collectively placed in a binder (one binder for each field season), and stored in a safe area where they can be referenced at a later date if any questions arise.

Data Analyses

The statistical methods for assessing long-term population trends based on count data at breeding ponds still need to be resolved. Biologists and statisticians from the US Fish and Wildlife Service and US Geological Survey are currently working on statistical methods appropriate for these types of data and methods will be better developed within the next 5 years. Since it will be several years before this protocol produces enough data to begin to attempt trend analysis, a lack of certainty regarding analytical approach is not problematic.

One potential technique would be a variation of the route-regression analyses currently used by biologists to analyze Breeding Bird Survey data collected from over 3000 routes across North America, termed here the stop-regression technique. In this case, data collected at individual breeding ponds (or stops along survey routes) could be considered equivalent to routes on BBS surveys, as breeding ponds represent individual, independent 'populations'. Thus, population trends on the Cape can be estimated as the weighted average of the stop trends. A linear model is used to estimate the trend for each stop (*i.e.*, breeding pond). The model is fit using linear regression on the logarithm of (counts + 0.5). The estimate of $\ln(b_0)$ is back-transformed to estimate (b_0) at each stop (*i.e.*, the slope is calculated for each individual stop). The back-transformation is $\exp(\ln[b_o]-0.5\text{variance}[\ln\{b_o\}])$.

The overall trend is calculated as the weighted mean of the stop (breeding pond trends), where the overall trend equals the sum of, for all stops, the marginal mean count for the stop times the estimated stop trend times a weighting factor divided by the sum, of for all stops the marginal mean count for the stop times a weighting factor, where the weighting factor at each stop is inversely weighted by the relative variance of the stop trend estimate. Regression analyses could be completed using SPSS or SAS.

An alternative strategy is to using the Occupancy Estimation routine in Program MARK (White and Burnham 1999), which was based on research by MacKenzie *et al.* (2002).

This analysis currently allows one to use information-theoretic methods (Anderson *et al.* 2000) to estimate detection probabilities (p) and the proportion of wetlands occupied (Ψ). There are currently no trend estimates available in the statistical package, but it should be added in the near future.

Egg Mass Counts

Egg mass counts will be the primary means of monitoring populations of spotted salamanders (*Ambystoma maculatum*) and wood frogs (*Rana sylvatica*). Egg mass counts are a very effective means of determining the size of these breeding populations (Crouch and Paton 2000). They take a relatively short period of time to conduct and are repeatable among years.

Egg mass counts conducted at CACO are designed to provide data for use at both the local CACO level and as part of a broader regional program of vernal pond amphibian monitoring coordinated by the USGS known as ARMI (Amphibian Research and Monitoring Initiative). While both track long terms trends in occurrence and size of breeding populations, ARMI also looks at these parameters with respect to road proximity, and at the abundance and occurrence of species within a group of adjacent ponds. Consequently a total of 20 sites will be monitored, with three counts plus a fourth "survey" (to detect species presence).

Sampling Sites

Since spotted salamanders and wood frogs breed primarily in vernal ponds, the 20 sampling sites will be vernal ponds (Table 26, Figures 13, 14, 15). From a large group of vernal ponds, a smaller group of ponds were selected based on meeting at least one of the following criteria: existence of historic egg mass count data; site of ongoing hydrological monitoring; site of anuran call count survey; proximity to road. Sites were selected randomly from this group, with adjacent sites added in accordance with ARMI protocols (Jung 2002)

Table 26. List of egg mass count sites for USGS and URI protocols. E: Eastham, W: Wellfleet, T: Truro, P: Provincetown.

Site	Town	USGS Site	USGS Type	Road Proximity	Easting	Northing
E11	E	yes	adjacent to E4	Far	420196	4633918
E11east	E	yes	adjacent to E4	Far	420262	4633908
E2	E	yes	adjacent to E4	Far	420291	4633806
E21	E	yes	focal	Close	420069	4632956
E22	E	yes	adjacent to E21	Far	419936	4632907
E3	E	yes	adjacent to E4	Far	420420	4633929
E4	E	yes	focal	Far	420298	4634044
E5 main	E	yes	adjacent to E4	Far	420071	4634077
E5a	E	yes	adjacent to E4	Far	420077	4633994
E6	E	yes	adjacent to E4	Far	420225	4634328
E7	E	yes	adjacent to E4	Far	420337	4634250
E8	E	yes	adjacent to E4	Far	420533	4634102
P04	P	no	none	Close	401405	4657852
T01	T	no	none	Far	412586	4648436
T15	T	no	none	Far	414104	4646568
W1	W	no	none	Far	411106	4645239
W15	W	yes	focal	Far	418064	4641832
W18	W	no	none	Far	418750	4640381
W6	W	yes	focal	Close	415966	4645301
W7	W	no	none	Far	417431	4644996

Figure 13. Egg mass count sites in Eastham.

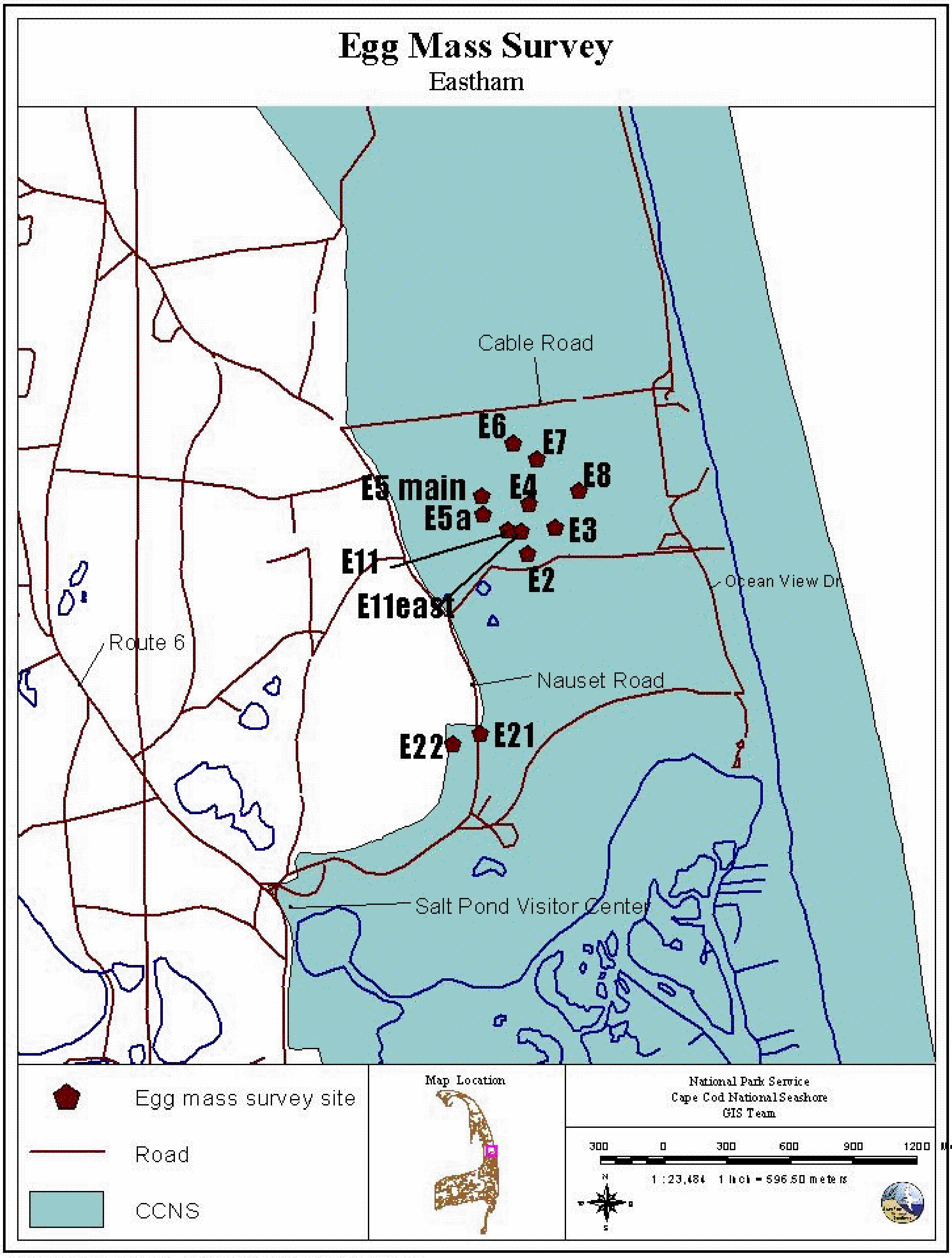


Figure 14. Egg mass counts sites in Wellfleet and Truro.

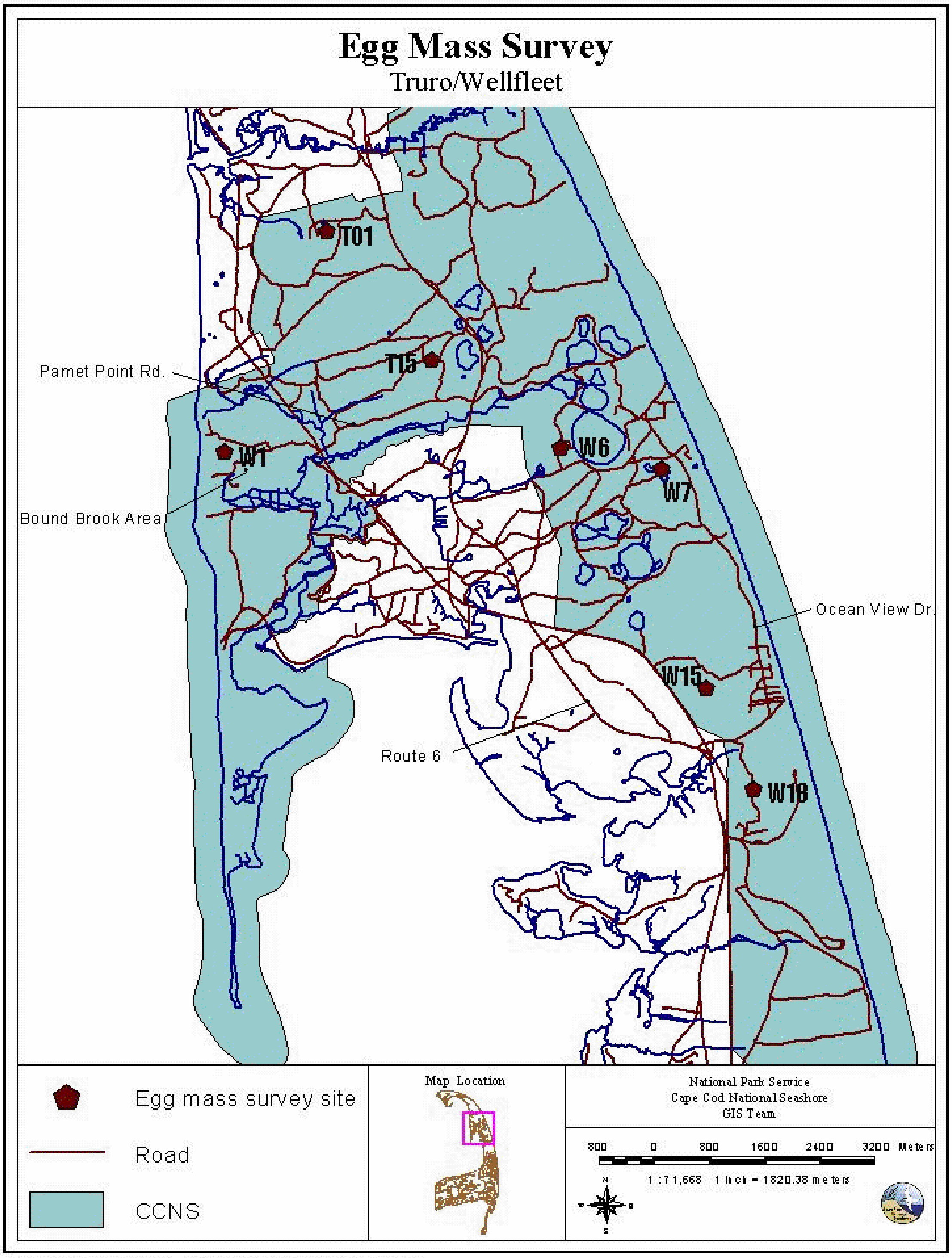
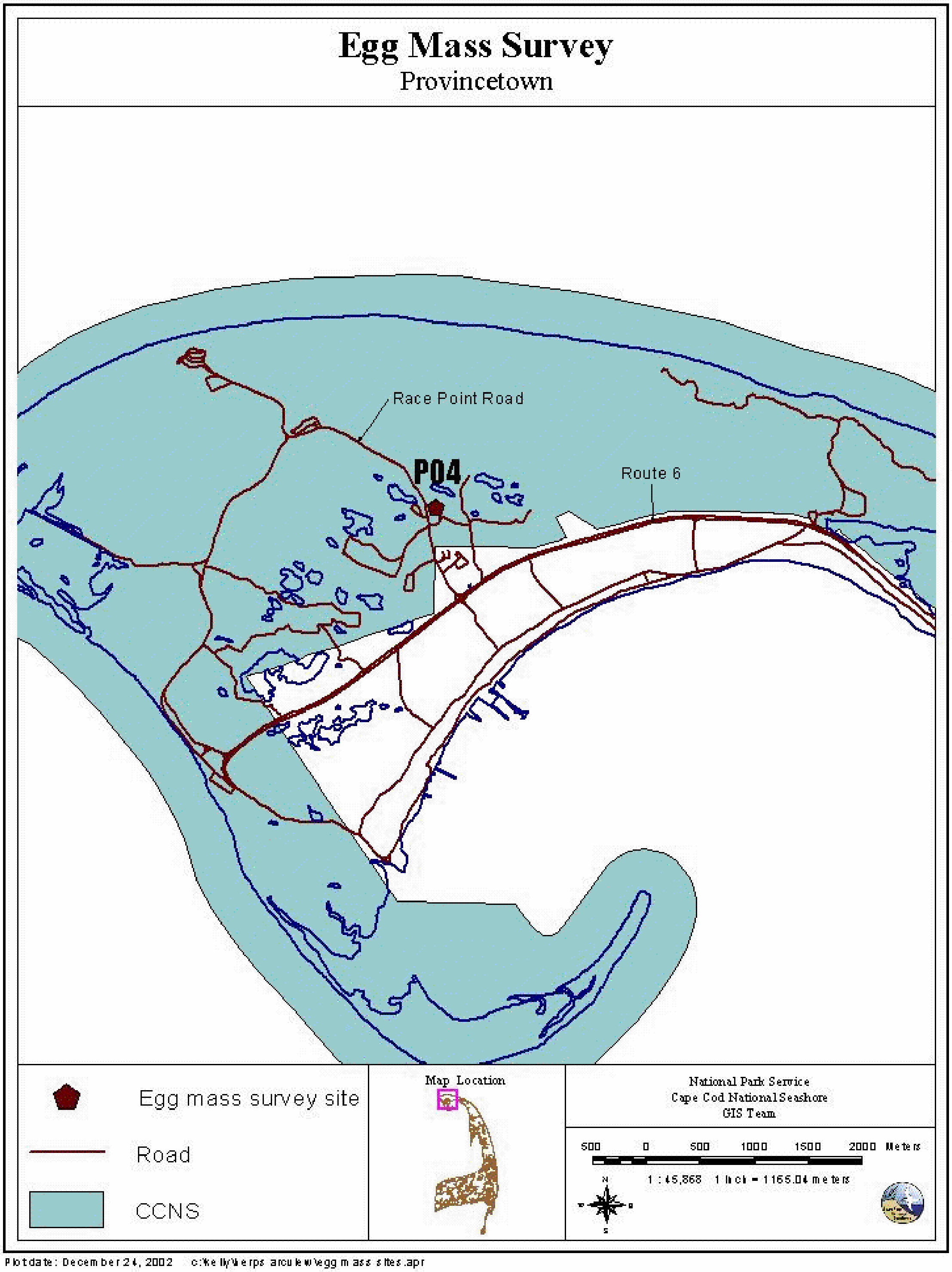


Figure 15. Egg mass count sites in Provincetown.



Sampling Schedule

A total of three Egg Mass Counts, plus a forth "survey" (to record presence/absence of amphibian species) will be conducted from early to mid spring. While exact dates will vary each year due to the calendar and daily weather conditions, the timing of counts will be as follows:

Count 1 – during last week of March-first week of April
Count 2 – middle two weeks of April
Count 3 – last week of April, first week of May
Survey – second week of May

Order of Sampling

Since wood frogs tend to breed earlier than spotted salamanders, and wood frogs occur in the southern end of the park, within each count replicate ponds will be sampled from south to north. This order will be repeated each year.

Time of Sampling

Counts will be conducted in the daytime, preferably when the sun is overhead. No counts will occur within 2.5 hours of sunrise or sunset.

Time Required for Field Work

Each count replicate is anticipated to require the equivalent of three workdays for fieldwork, with time to sample sub-groups of ponds estimated as follows:

E21-E22: 3 hrs
E4 and adjacent areas: 8 hrs
Wellfleet/Truro sites: 8-10 hrs
P4: 1 hour

Sampling Conditions

Sampling will be daytime only, on days when there is no precipitation, cloud cover is less than 50%, and glare from the sun is not significant. Samplers will wear polarized sunglasses to minimize glare.

Within the period for count 1, weather conditions will be variable. If pond water is cold ($<8^{o}C$) and there has been no substantial rain, count 1 should be deferred as long as possible/necessary within its "window" until environmental conditions are appropriate.

Environmental Data

Data on air and water temperatures (F^{o}), wind, and sky will be collected at the start of each count of each pond and entered into the field data sheet (Table 27). For each pond, the point of maximum depth will be marked with a permanent stake, and maximum water depth recorded there on each of the four visits.

Data on pond water quality (e.g. pH, alkalinity, chloride, color, conductivity, and tannin-lignin) will be collected once (in early spring) during the daytime as part of a broader program of water quality monitoring.

Habitat Data

At the four ponds to be included as "focal ponds" in the USGS ARMI program (E4, E21, W6, and W15) categorical data on habitat variables within and adjacent to the pond will be collected and recorded on a data sheet in accordance with Jung (2002).

Equipment List

During each egg mass count the observer(s) should bring with them to the site:

- (1) pair of chest waders for each observer
- (1) waterproof field book and/or data sheet (Table 27)
- (several) graphite mechanical pencils
- (1) waterproof meter stick
- (1) air thermometer
- (1) water thermometer
- (1) water resistant watch and/or stopwatch
- flagging on 36" wire
- rolls of flagging tape
- medium point, black permanent marking pen

Counting Protocol

Prior to data collection, the pond shoreline will be flagged at points to mark north, south, east, and west.

Data on environmental variables will be collected upon arrival at the pond, except that maximum depth will be recorded at whatever point in the count workers are at a pond's deepest point. Making an additional pass into the pond to collect depth data should be avoided.

For the 16 ponds that are not "ARMI Focal Ponds" the observer(s) will begin the count at one location along the edge of the pond (if possible at one of the cardinal direction points) and will walk systematically (and carefully) throughout the pond, making sure to survey the entire pond. Upon the discovery of each egg mass or group of masses (locus), the observer will identify the species, count and record the number of egg masses of each morph (clear or white) at that locus, give that locus an alpha-numeric identifier, (W for wood frog, S for spotted salamander followed by a number starting at #1 and working up consecutively), determine the quadrant of the pond that locus lies in (NE, SE, SW, or NW), and mark them by placing one 36" wire surveyor flag (alternatively, use flagging to mark egg masses, although woody vegetation is generally not available near egg masses to attach flagging). On the flagging write (with permanent marker) the locus identifier, total number of egg masses at that locus, and the date on the flag adjacent to the locus and also record this information on the data sheet (Table 27).

Upon completion of the count at each site, when observers are confident they have sampled the entire pond, they will record the end time (military) and record any notes which they feel pertinent regarding the count (*e.g.* weather conditions, wildlife observations *etc.*).

During the second round of censuses at each site the observer(s) will begin at the same location as the prior count and follow the same techniques as mentioned prior. For each locus encountered that was previously identified (marked by a wire flag), the same locus number will be used, and the number of egg masses of each morph will be recounted. At the end of this count, the locus number, date, and count will be written on the opposite side of the flagging and recorded on the data sheet. For each new locus encountered the observer will follow the same procedure as previously mentioned, using a W or S to identify species then starting with locus #1A and working up consecutively by one each time following each number with an “A". The same procedure will be followed for the third count, with any new loci recorded on this third visit labeled consecutively starting as “1 B”. When all of these data have been recorded the observer(s) will remove all of the wire flags within the pond denoting egg mass loci.

For the four ARMI Focal Ponds, the same procedure for labeling loci will be followed. However, as detailed in Jung (2002) a double observer method will be used for the count, with observers switching roles halfway around the pond. Count data will be recorded on two separate data sheets, one for the ARMI program and the other the same as used elsewhere in this protocol.

<u>Data Entry</u>

For each egg mass count conducted, a field data sheet (Table 27) will be filled out, and from that the data will be transcribed into MS Excel (or Access) spreadsheet. Observers will enter the data into the database no later than 24 hours after the completion of the count in order to minimize any confusion as to the data that were collected. All counts from a season will be entered into the same spreadsheet and will be proofed before analyses are initiated. Data should be saved to the computer's hard drive and at least two separate disks, with disks stored in separate locations. All raw data sheets will be three-hole punched, collectively placed in a binder (one binder for each field season per survey technique), and stored in a safe area where they can be referenced at a later date if any questions arise.

Data Analysis

As with call count data, the analysis of egg masses count data will evolve tremendously over the next decade. Thus it is imperative that observers retain a standardized methodology to quantify egg mass counts. The advantage to egg mass counts is that the dependent variable in this model (egg mass counts) is a quantifiable variable that will vary continuously among years. The unit of measure with this protocol is the sum of the maximum number of egg masses at each locus. For example, if locus S1 had 10 egg masses at count#1, 25 egg masses at count#2, and 28, and count#3, then the maximum for S1 would be 28. The sum of the maximum number of egg masses at each locus is used as the estimate of total number of egg masses in that pond that year. As with call count data, linear regression can be used to estimate long-term population trends. The model will be fit using a linear regression on the logarithm of (counts + 0.5), and then the estimates of $\ln(b_0)$ will be back-transformed to estimate the annual rate of population change. The back-transformation is $\exp(\ln[b_o]-0.5\text{variance}[\ln\{b_o\}])$. The average rate of change could be used to estimate breeding population trends of spotted salamanders at Cape Cod, or this estimate could be weighted by overall population size at each pond to estimate overall breeding population trends. Regression analyses could be completed using SPSS or SAS.

Table 27. Data sheet for egg mass counts.

Egg Mass Counts – Field Data Sheet

Page ____ of ____

Observers: ______________ Site Name: _______________ Date:____________
Start Time: _____________ End Time: _______________ Water Depth (max, cm):______________
Air Temp: _____________ Water Temp: _____________ Sky: ________ Wind: ___________
Notes: ____

							Depth (cm)		
Species*	**Locus #**	**Quadrant**	**Clear Masses**	**White Masses**	**Total Masses**	**Stage**	**Egg Mass**	**Water**	**Attachment Substrate**

* SPSA= spotted salamander WOFR= wood frog GRFR= green frog BUFR= bullfrog PIFR= pickerel frog ESTO= Eastern spadefoot toad

Nocturnal Road Surveys

While Nocturnal Road Surveys are not recommended as a general monitoring method, this method may be very useful for monitoring of Eastern Spadefoot Toads in the Province Lands. However, its utility requires further study. This Nocturnal Road Survey protocol is included to provide a starting point for further development of this approach at CACO, and to provide guidance to other coastal parks that may be considering it.

Site Selection

There are four different routes (Provincetown, Eastham, Wellfleet, and Truro) where nocturnal road surveys could be conducted.

Provincetown Route: The observer(s) will drive north on Route 6 until the first intersection with Provincelands Road. At this intersection, the observer(s) will turn right onto Provincelands Road and the survey will begin. The observer will follow Provincelands Road until they get to the Provincelands Visitor Center where they will drive through the parking lot. After driving through the parking lot the observer(s) will continue on Provincelands Road in the same direction as before and will bear to the right at the intersection with Airport Drive. They will follow Airport Drive all the way to the end where it ends in the beach parking lot, where they will drive through the parking lot and then follow Airport Drive in the opposite direction as before. At the intersection with Provincelands Road they will bear to the right onto Provincelands Rd. and follow this all the way down to the Herring Cove Beach Parking Lot. Here the observers will take a right and enter the parking lot. At the T-intersection at the stop sign the observer(s) will take a left and drive through the south parking lot. After driving around the parking lot the observer(s) will return to the intersection with Provincelands Road. They will take a left here and follow the previous route in reverse, with one exception: when the observer(s) reach the Hatches Harbor Dike Road parking lot they will pull into the lot and turn off the vehicle. Then the observer(s) will walk down the Hatches Harbor Dike Road 100 m. Then the observer(s) will turn around and walk back to the vehicle along the same route. Once at the vehicle the observer(s) will continue on the route, taking a left out of the parking lot, following Provincelands Road until the intersection with Airport Drive, following Airport Drive to the parking lot, drive around the lot, following Airport Drive in the opposite direction until the intersection with Provincelands Road, take a left onto Provincelands Road, follow until the Provinceland Visitor Center parking lot where they will drive around the parking lot, then take a left back onto Provincelands Road until the intersection with Route 6 where the route will end.

Eastham Route: Following Route 6 south, the observer(s) will take a left onto Nauset Road at the light, and once on Nauset Road the survey will begin. The observer(s) will follow Nauset Road past the Salt Pond Visitor's Center and will follow it all the way to the intersection with Ocean View Drive near the entrance to Coast Guard Beach. Here the observer(s) will turn left, onto Ocean View Dr., and follow this all the way to the stop sign at the intersection with Cable Road. Here the observer(s) will turn left, onto Cable

Road, and follow this all the way to the stop sign at the intersection with Nauset Road. The observer(s) will take a left onto Nauset Road here and follow it all the way to the end to the stop sign at the intersection of the other Nauset Road where this route will end.

Wellfleet Route: Observer(s) will drive south on Route 6 until the light at the intersection with the Marconi Station Road. They will take a left here, onto Marconi Station Rd. and the survey will begin. The observers will follow this road all the way to the end where it turns into the parking lot. They will take the loop around the parking lot and return onto Marconi Station Rd. and follow it in the opposite direction. They will follow the road until the intersection with Headquarter's Road where they will take a right. They will then follow Headquarter's Road to the end where it turns into the parking lot for the Marconi Station Site. They will continue through the parking lot until it loops back onto Headquarter's Road which they will take in the opposite direction until the intersection with Marconi Station Rd. At this intersection the observers will take a right and follow Marconi Station Rd. until it intersects with Route 6 at the set of lights. This concludes this first portion of the route. The observer(s) will then take a right at the lights, and head north on Route 6 until the intersection with Lecount's Hollow Road. The observer(s) will take a right onto Lecount's Hollow Rd. and the second portion of this route will begin. They will follow this road all the way until it intersects with Ocean View Drive where they will take a left. They will follow this road all the way to the stop sign at the intersection with Gross Hill Road. At this intersection they will go right onto Gross Hill Road and follow this all the way to the end at the parking lot to Newcomb Hollow Beach. The observer(s) will take a loop through the parking lot and then return on Gross Hill Road and follow it all the way to the intersection with Gull Pond Road. The observer(s) will take a right at this intersection and follow Gull Pond Road all the way to the intersection with Route 6 where this route will end.

Truro Route: Observer(s) will drive north on Route 6 until the exit for the Pamet Roads. Here they will take a right and at the end of the ramp will take a left at the stop sign onto North Pamet Road beginning the survey. They will follow North Pamet Road all the way to the parking lot for the Upper Pamet calling survey site. Then they will turn around and follow North Pamet Road in the opposite road until the stop sign at the T-intersection with South Pamet Road. Here the observer(s) will take a left onto South Pamet Road and follow it all the way to the end to the parking lot for Ballston Beach. They will take a loop through the parking lot here and then return on South Pamet Road in the opposite road until the intersection with North Pamet Road where the route will end.

<u>Equipment List</u>

During each nocturnal survey the observer(s) will have with them:

- (several) flashlights and/or headlamps
- (ample) backup flashlight/headlamp batteries
- (1) waterproof field notebook and/or data sheet (Table 28)
- (several) graphite mechanical pencils
- (1) air thermometer

- (1) weight scale (precision 0.1g)
- (several) plastic rulers (precision 1mm)
- (several) collection containers with formalin
- (several) small toe-clipping scissors
- (1) digital 35mm camera
- (1) reflective vest for each observer
- (1) flashing belt for each observer
- (1) flashing yellow light for roof of vehicle (optional)
- (1) watch/stopwatch

Protocol

Road surveys will take place from 15 May through 1 September as those are dates when spadefoot toads (*Scaphiopus holbrookii*) are most active. Surveys will be conducted on all nights when there is significant precipitation or on nights following a day of heavy rain. Particular emphasis will be placed on nights with very heavy rainfall and/or during thunderstorms as these are nights when this species is most active. Observers will begin the surveys one hour after sunset and continue until one hour after the rain stops or until sunrise (whichever one comes first). Observers will follow the routes as mentioned in the site selection portion of this technique description. Upon initiation of the route the observers will reset the vehicle's trip odometer. Thereafter, anytime observers turn on to a different road or enter into a parking lot they will reset the odometer as to get an accurate location when they encounter any amphibian on the roadway so they can reference that location and return to it to GPS it at a later date. Observers will use either a government vehicle (NPS) or another such vehicle and must make sure to have some official documentation with them that explains what exactly they are doing and that gives them authority to be doing such activities in case of an encounter with law enforcement (NPS and/or local/state law enforcement officials). Observers will have with them one waterproof notebook which they will record all of the information of the survey. Upon initiation of the survey the observer(s) will record the observer(s) name(s), route name, date (mm/dd/yy), start time (military), time the rain began (military), the wind and sky codes (Tables 22 and 23) at the start, and any pertinent notes as to weather conditions or any information that may be useful. Then the observer(s) will drive the previously determined route (as described above). Observers will drive no faster than 10 mph while surveying the route as to be sure to maximize visual observation of amphibians on the roadways. Upon each encounter of an amphibian on the roadway observers will pull to the side of the road, turn on the vehicle's hazard lights (or flashing yellow light on roof of vehicle if present), put the car in park and, after assuring that it is safe from any traffic, observers will get out of the vehicle wearing their reflective vest (and flashing belt), and move as quickly as possible to the amphibian observed and pick it up off of the road. The observer will then move back off the side of the road to the vehicle where data will be recorded. Once back at the car the observer will record the time (military), location (mileage since the last odometer reset along with stating where the odometer was reset or some other precise location reference that will allow the observer to return at a later date and GPS the point), the direction the individual encountered was heading (*e.g.* heading westward across the road, heading toward Gull Pond etc.), species, age (metamorph, juvenile, or adult), sex, and whether the individual encountered was dead or alive. If the individual is found dead on the road, the observers are to collect the individual and place it in a jar with formalin and a label with observer name, date (mm/dd/yy), location, and species. Observers will measure the snout-vent length (to nearest mm) of every live individual with a plastic ruler, and in the case of caudates, a total length measurement (tip of snout to the end of the tail; nearest mm) will also be recorded. Every live individual encountered will also be weighed (0.1g) and observers will record whether or not the individual was toe-clipped prior, and in the case of toe-clipped individuals the year of the toe-clip will be recorded. If an individual is found not to already have a toe-clip they will toe-clip the animal. We recommend one digit be clipped, with a unique digit clipped each year. Digits are numbered from 1-5, with the innermost (proximal) digit being number 1. Digits on front limbs are numbered from 1-4, again with the proximal digit being number 1. Male spadefoot toads are identified by the presence of black excrescences (patches) on digits 1-3 on front limbs (see Wright and Wright 1949: p125). Along with these previous data recordings any pertinent notes that may be of interest will be recorded for each individual.

Upon completion of the road survey the observers will record the end time (military), the time the rain ended (military), the sky and wind codes (Tables 20 and 21) at the end of the survey, and also any notes that may be pertinent. After appropriate data are recorded, individuals are placed off the side of the road in the direction they were determined to be moving when first encountered.

Data Entry

For each nocturnal road survey conducted a field data sheet (Table 28) will be filled out, and from that the data will be transcribed into MS Excel (or Access) spreadsheet (example headers are given in Table 30). Observers will enter the data into the database no later than 24 hours after the completion of the survey in order to minimize data entry errors. All surveys from a season will be entered into the same spreadsheet and will be proofed before analyses are initiated. Data will be saved to at least one computer's hard drive and at least two separate disks. All raw data sheets will be three-hole punched, collectively placed in a binder (one binder for each field season per survey technique), and stored in a safe area where they can be referenced at a later date if any questions arise.

Data Analysis

As with call survey and egg mass count, the analysis of road survey data will evolve tremendously over the next decade. The count data used in this analysis is the maximum number of individuals detected in each 1-km segment of road surveyed during nocturnal road surveys. As a first step, the analyst will have to determine the total number of individuals detected in each 1-km segment of road surveyed. Then data from multiple nights of road surveys within a year should be pooled to determine the maximum number of individuals detected in each 1-km segment of road. The mean number of individuals per km of road (based on these maximum count data) will then be count metric used for subsequent analyses. Thus it is imperative that observers retain a standardized methodology to quantify amphibians crossing road. The advantage to roadside counts is that the dependent variable in this model (number of individuals detected crossing roads) is a quantifiable variable that will vary continuously among years. As with call survey data, linear regression can be used to estimate long-term population trends. The model will be fit using a linear regression on the logarithm of (counts + 0.5), and then the estimates of $\ln(b_0)$ will be back-transformed to estimate the annual rate of population change. The back-transformation is $\exp(\ln[b_o]-0.5\text{variance}[\ln\{b_o\}])$. The average rate of change could be used to estimate population trends of roadside trends at Cape Cod.

Table 28. Data sheet for nocturnal road surveys.

Nocturnal Road Surveys – Field Data Sheet

Page ____ of ____

Observers: ________________ Route Name: __________________ Date: ________________
Start Time: ______________ End Time: _________________
Time Rain Began: __________ Time Rain Ended: _____________
Sky Begin: ____ Sky End: ____ Wind Begin: ____ Wind End: ____
Notes: ____

Time	Location (Mileage)	Direction Indiv. Heading	Species*	Age	Sex	Dead/ Alive	Collected ?	SVL (cm)	TL (cm)	Mass (g)	TC	Notes

*SPSA= spotted salamander FTSA= four-toed salamander RBSA= redback salamander RSNE= red-spotted newt WOFR= wood frog GRFR= green frog BUFR= bullfrog PIFR= pickerel frog SPPE= spring peeper FOTO= Fowler's toad ESTO= Eastern spadefoot toad

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APPENDICES

Appendix I. Location of ponds (UTM coordinates) surveyed during manual anuran call survey in the 2001 field season at Cape Cod National Seashore.

I. Route One (Wellfleet and Eastham) UTM coordinates of station locations:

1. Grassy Pond, Shallow Kettle Pond	e0416901	n4643453
2. W20, Deep Kettle Pond	e0416916	n4643087
3. W7, Vernal Pond	e0417431	n4644996
4. Kinnacum Pond, Deep Kettle Pond		
5. W18, Vernal Pond	e0418750	n4640381
6. W17, White Cedar Swamp	e0418675	n4644050
7. Motel Bog, Cranberry Bog	e0418091	n4637076
8. E9, Vernal Pond	e0420056	n4633659
9. E4, Vernal Pond	e0420298	n4634044
10. E16, Vernal Pond	e0420925	n4634371
11. E18, Buttonbush Swamp	e0419354	n4632317
12. E15, Red Maple Swamp	e0416780	n4630402

II. Route Two (Wellfleet and Truro) UTM coordinates of station locations:

1. W6, Vernal Pond	e0416901	n4643453
2. Gull Pond, Deep Kettle Pond	e0416120	n4645357
3. Herring Pond, Deep Kettle Pond	e0416167	n4646070
4. Black Pond, Riparian Marsh	e0415083	n4646131
5. T14, Vernal Pond	e0414104	n4646568
6. T15, Vernal Pond	e0414263	n4646623
7. Snow Pond, Deep Kettle Pond	e0414887	n4646816
8. Upper Pamet River, River	e0415020	n4650306
9. Pamet Bog, Riverine Bog	e0414806	n4650581
10. Ballston Marsh, Riparian Marsh	e0415412	n4650231

III. Route Three (Provincetown) UTM coordinates of station locations:

1. Great Pond, Inter-dunal Pond	e0401178	n4657884
2. Lily P.South, Inter-dunal Pond	e0400801	n4657799
3. Lily P. Main Inter-dunal Pond	e0401061	n4657857
4. P15, Vernal Pond	e0401301	n4657742
5. P16, Vernal Pond	e0401342	n4657701
6. P4, Vernal Pond	e0401405	n4657852
7. P5, Dune Slack Pond	e0401414	n4658014
8. P8, Dune Slack Pond	e0400610	n4658421
9. P13, Dune Slack Pond	e0400256	n4658421
10. P6, Dune Slack Pond	e0399857	n4658332

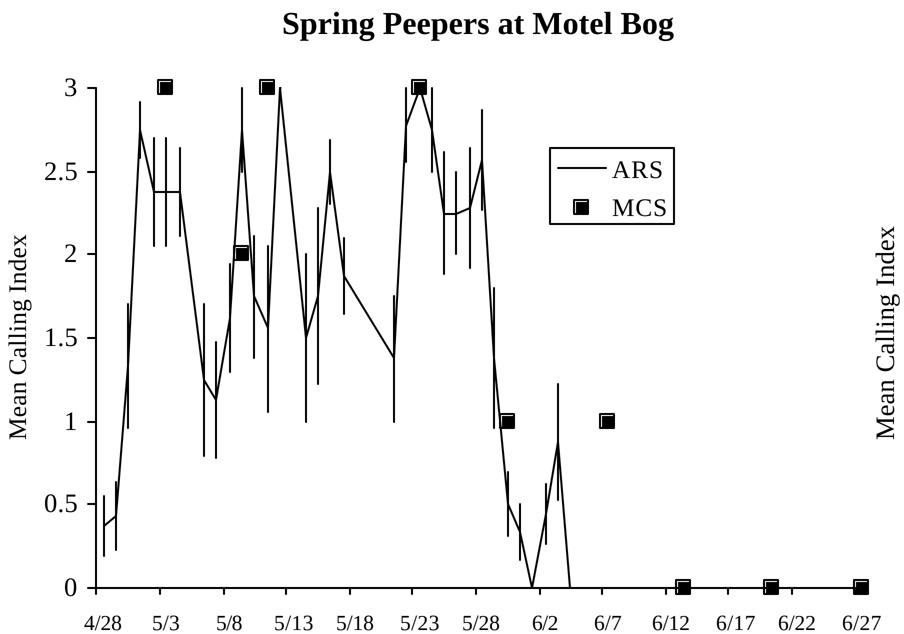
Appendix II. Habitat characteristics at ponds surveyed during the 2001 field season at Cape Cod NS.

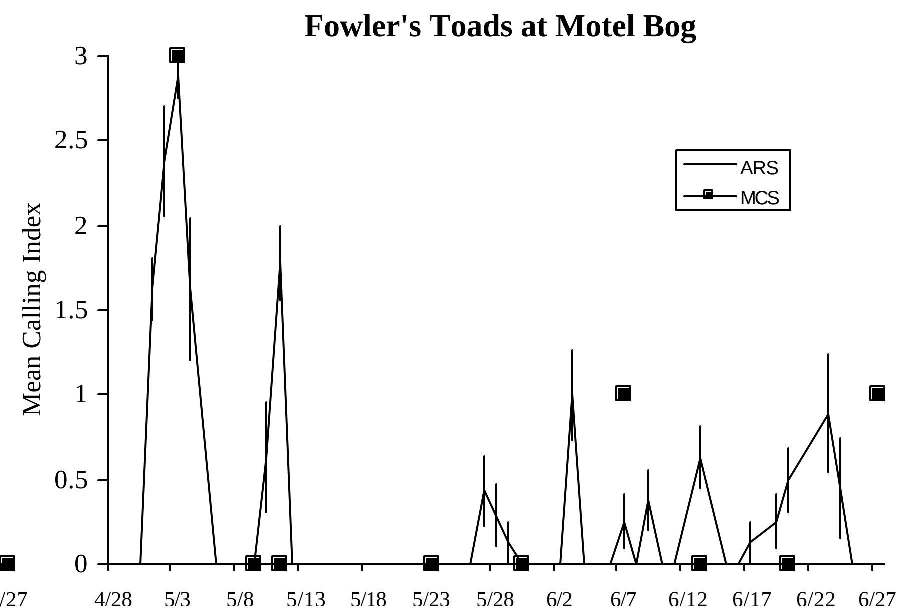
Site	Town	Habitat Type	Water Regime	Dominant Veg.	Dimensions (m2)
Ballston Marsh	Truro	RM	SPF	*Phragmites sp.*	
Black Pond	Wellfleet	RM	PF	open water	
E4	Eastham	VP	SF (late)	*Dulichium arundinaceum*	1617
E9	Eastham	VP	SPF	*Juncus canadensis*	2800
E15	Eastham	S (red maple)	SF (early)		
E16	Eastham	VP	SF (late)		
E18	Eastham	S (shrub)	PF	*Cephalanthus occidentalis*	7345
Grassy Pond	Wellfleet	STP	PF	*Scirpus cyperinus*	7144
Great Pond	Provincetown	CPP	PF	*Pontederia cordata/* open water	
Gull Pond	Wellfleet	KP	PF	open water	
Herring Pond	Wellfleet	KP	PF	open water/ *Decodon verticillatus*	
Kinnacum Pond	Wellfleet	KP	PF	open water	13617
Lily Pond Main	Provincetown	CPP	PF	*Nymphaea odorata*	
Lily Pond South	Provincetown	CPP	PF	*Nymphaea odorata*	
Motel Bog	Wellfleet	DS/CP	SF (late)	*Vaccinium macrocarpon*	
Motel Bog	Wellfleet	S (shrub)	PF	*Decodon verticillatus*	
P4	Provincetown	VP	SF (early)	no vegetation	
P5	Provincetown	DS/CP	SF (late)	*Vaccinium macrocarpon*	13398
P6	Provincetown	DS/CP	SF (early)	*Vaccinium macrocarpon*	
P8	Provincetown	DS/CP	SF (early)	*Vaccinium macrocarpon*	
P13	Provincetown	VP	SF (late)	*Vaccinium macrocarpon*	
P15	Provincetown	VP	SF (early)	*Spiraea latifolia/* open water	
P16	Provincetown	VP	SF (early)	Downed woody debris	
Pamet Bog	Truro	RM	PF		15368
Snow Pond	Truro	KP	PF	Open water	46314
T14	Truro	VP	SF (early)	*Decodon verticillatus* / Smilax/ Sphagnum	759
T15	Truro	VP	SF (early)	Smilax	420
Upper Pamet	Truro	RM	PF	River	385
W7	Wellfleet	VP	SPF	*Decodon verticillatus*	2107
W12	Wellfleet	KP	PF	*Decodon verticillatus*	2232
W17	Wellfleet	S (Atlantic white cedar)	SF (early)	*Chamaecyparis thyoides*	
W18	Wellfleet	VP	SF (early)	*Vaccinium corymbosum/* open water	180

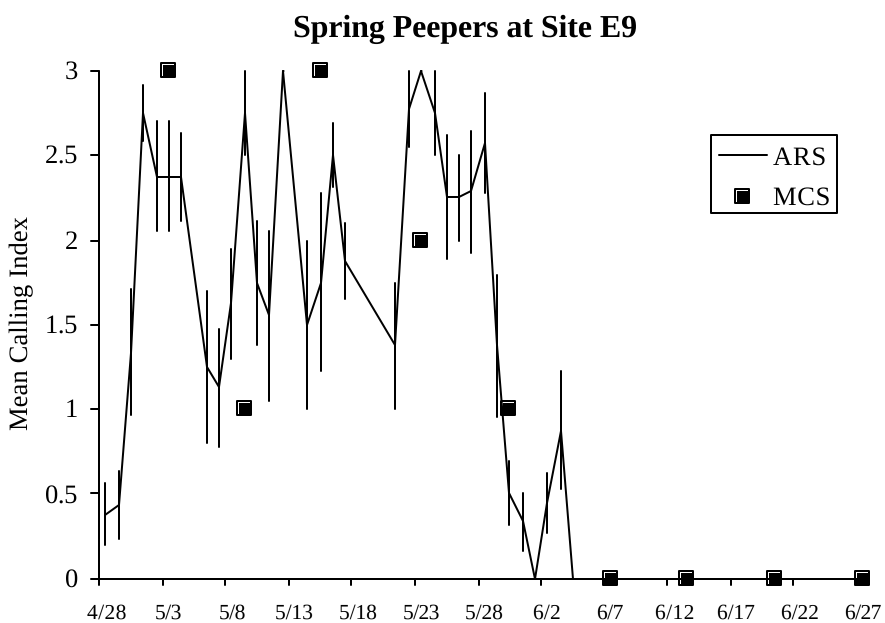
Habitat Type: RM=Riparian Marsh; VP=Vernal Pond; S= Swamp; STP=Shallow Kettle Pond; CPP=Inter-dunal Pond; KP=Deep Kettle Pond; DS/CP=Dune Slack

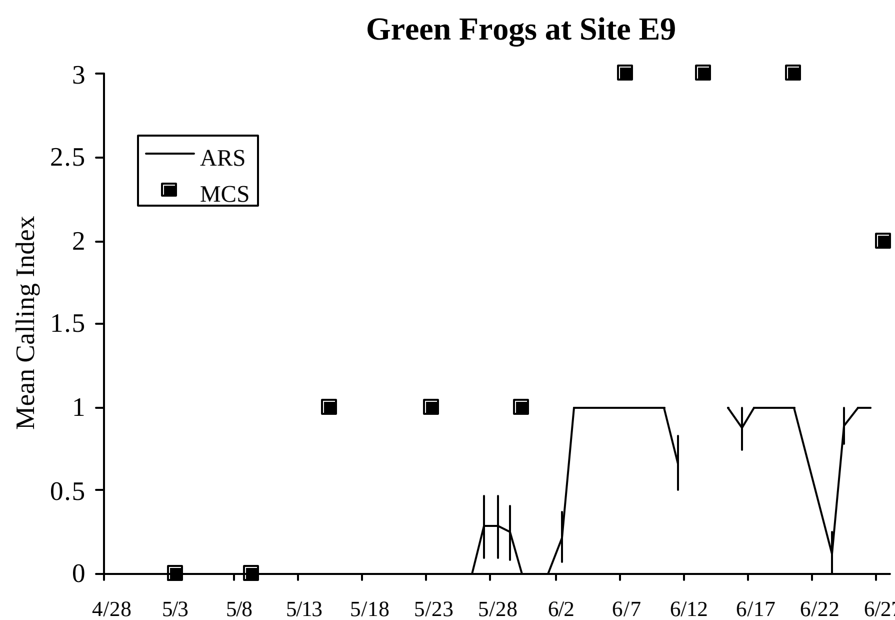
Water Regime: SPF=Semi-permanently Flooded; PF=Permanently Flooded; SF=Seasonally Flooded; (early)=dry by mid-June;(late)=dry after mid-June

Appendix III. Seasonal variation in calling intensity for selected species, comparing the relationship between automated recording systems (ARS) and manual call surveys (MCS) at some ponds

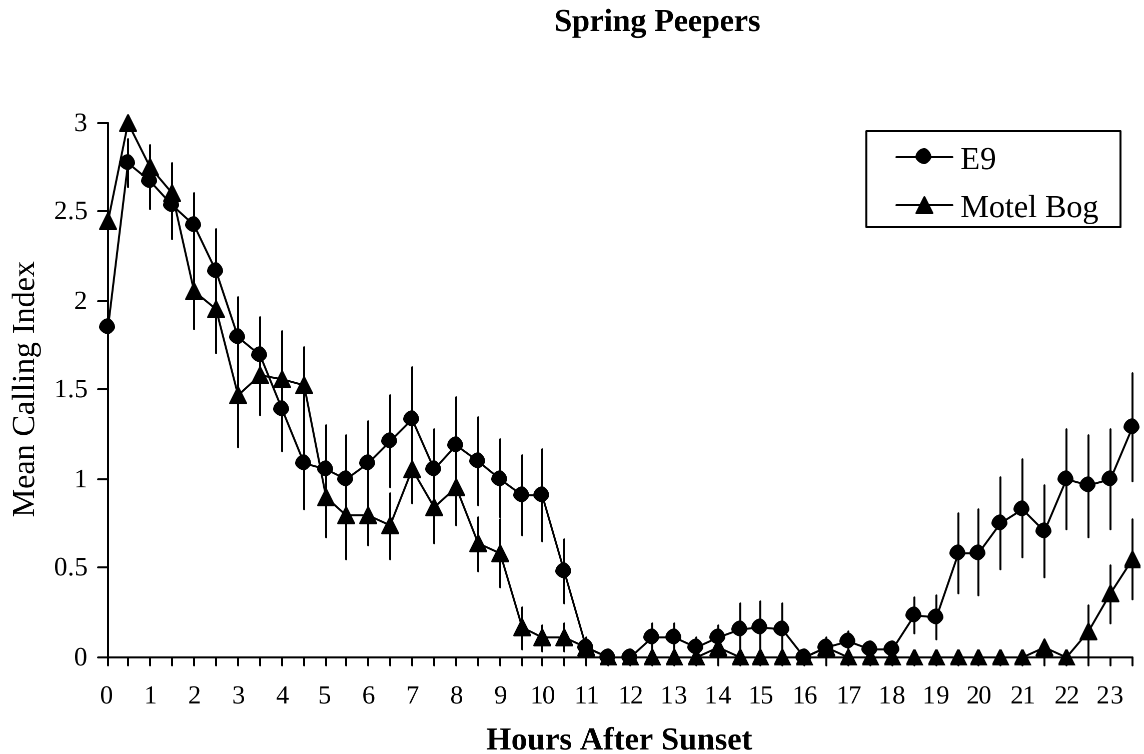


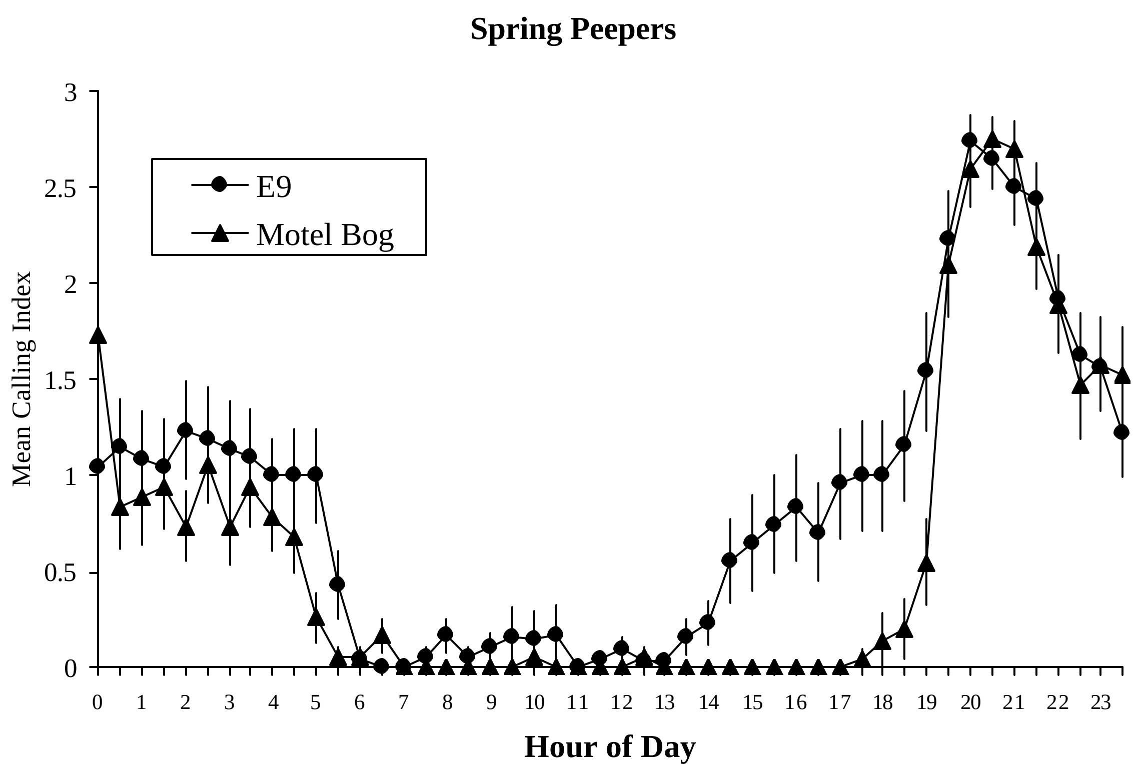


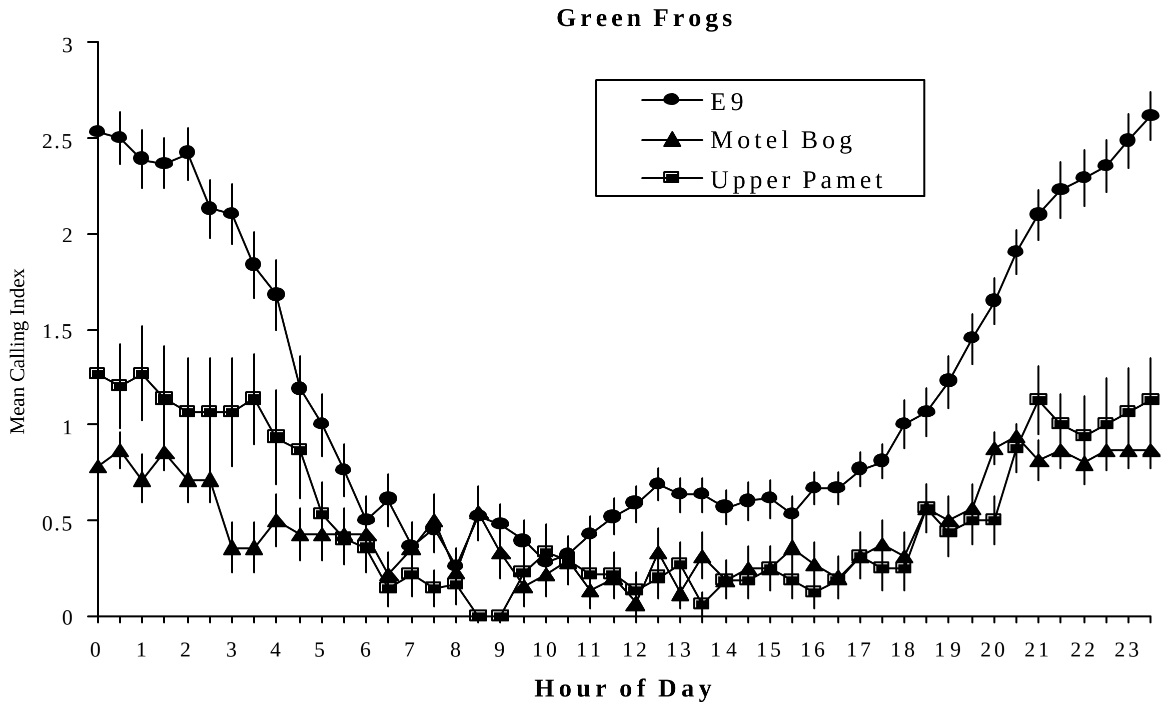


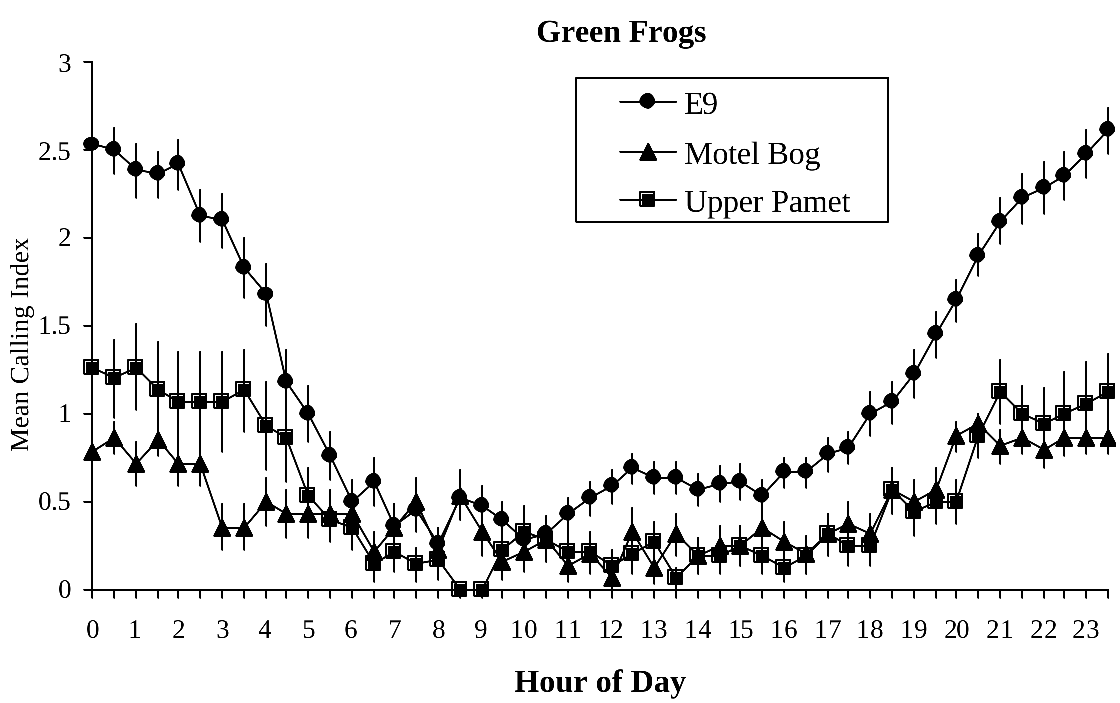


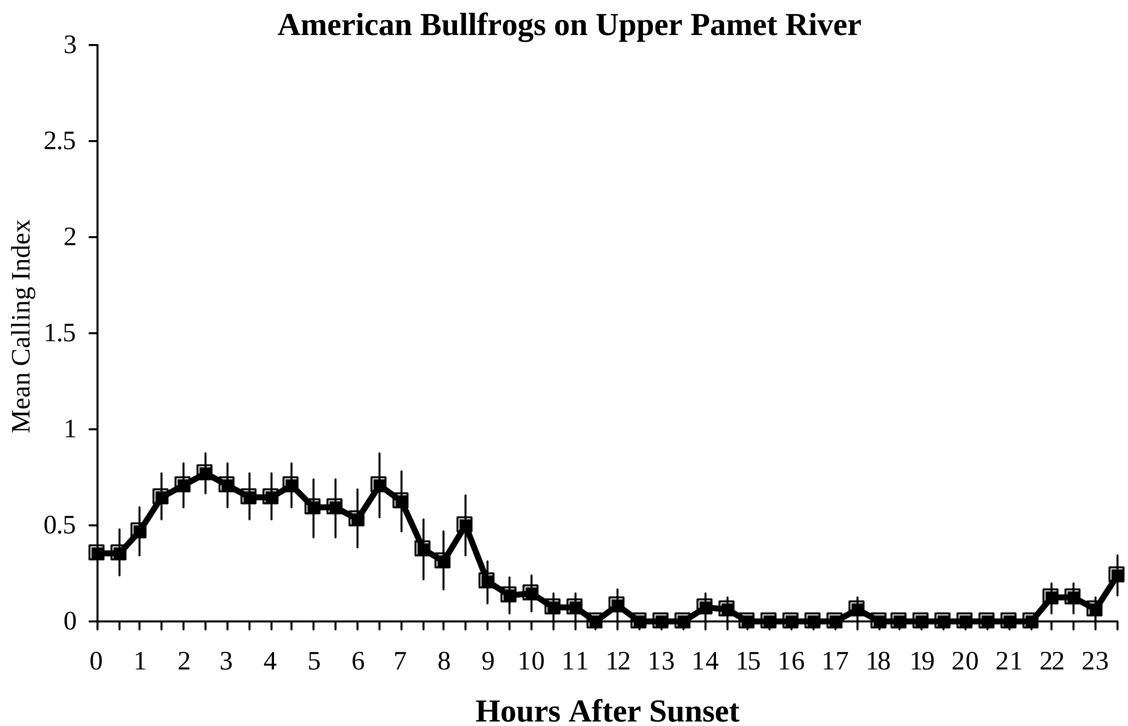
Appendix IV. Diel variation in calling intensity for anurans at Cape Cod NS based on ARS data. Shown are calling intensity in relationship to sunset and a 24-hour clock. Data from all ponds are shown.

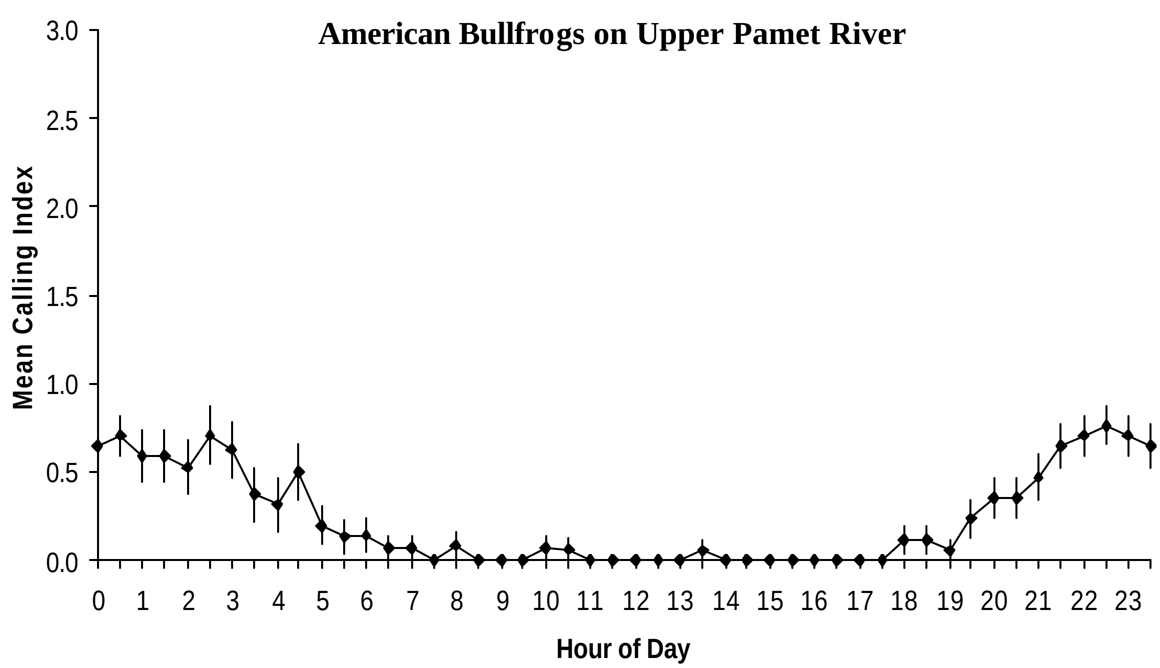


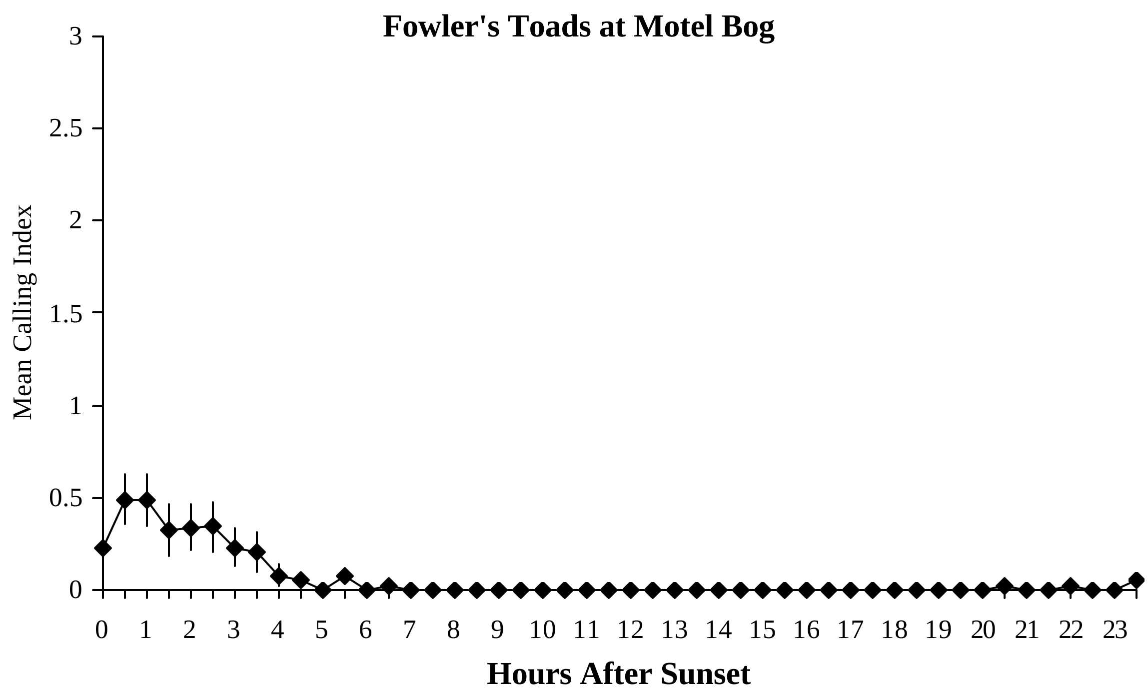


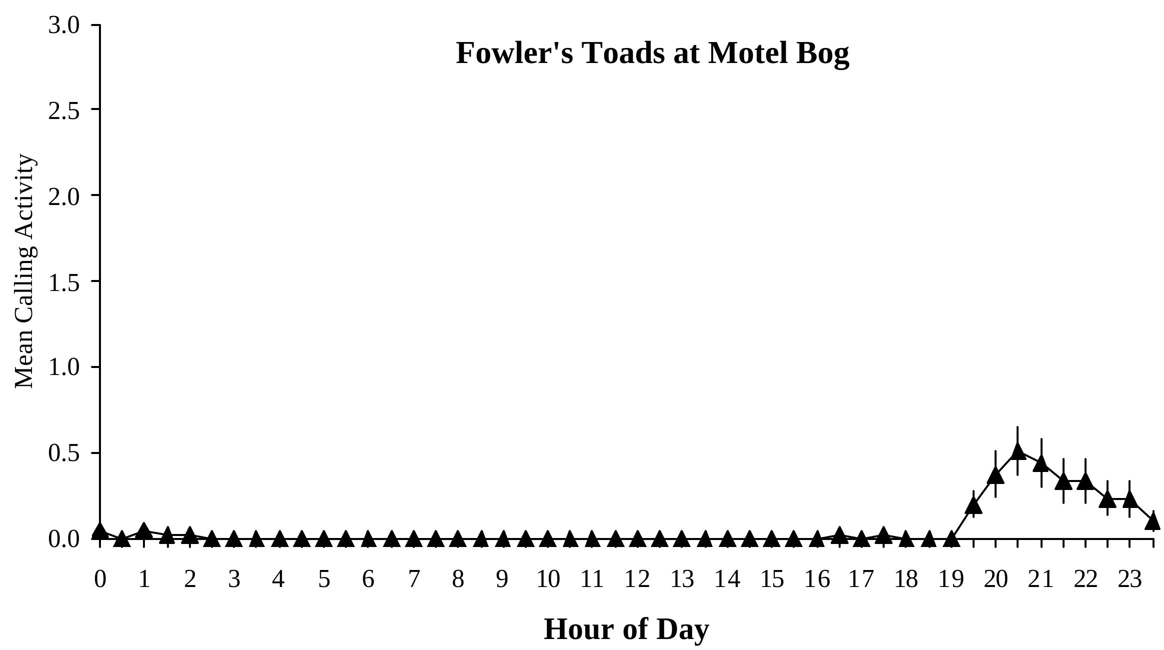

Green Frogs
E9
Motel Bog
Upper Pamet
Mean Calling Index
0
0.5
1
1.5
2
2.5
3
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Hour of Day

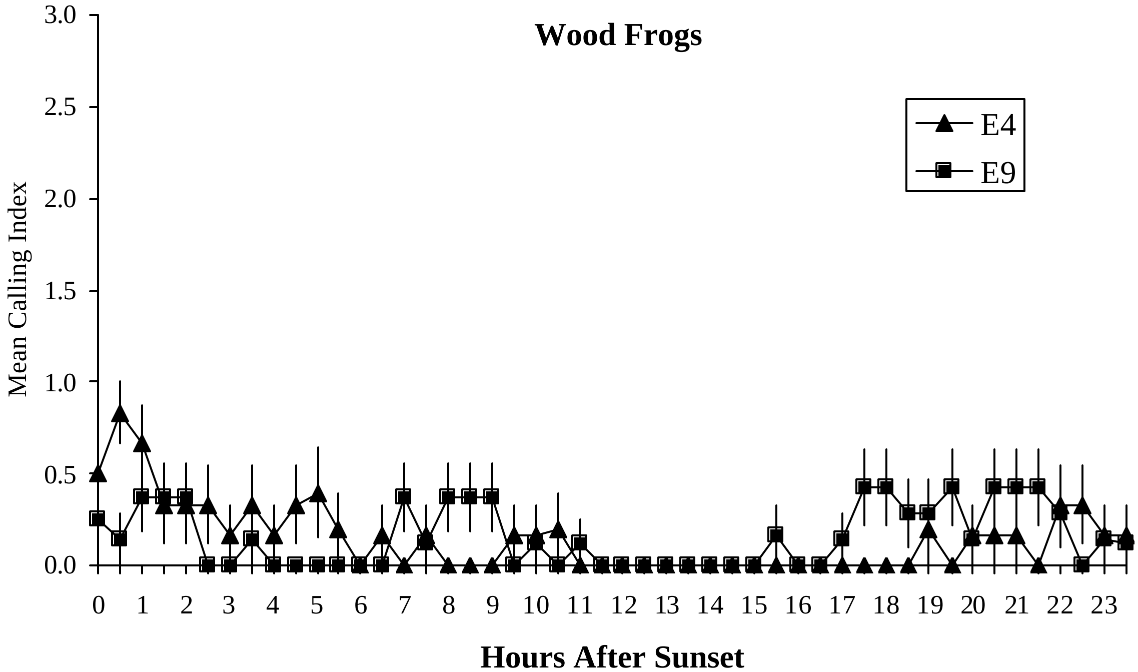

Green Frogs
E9
Motel Bog
Upper Pamet
Mean Calling Index
0
0.5
1
1.5
2
2.5
3
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Hour of Day

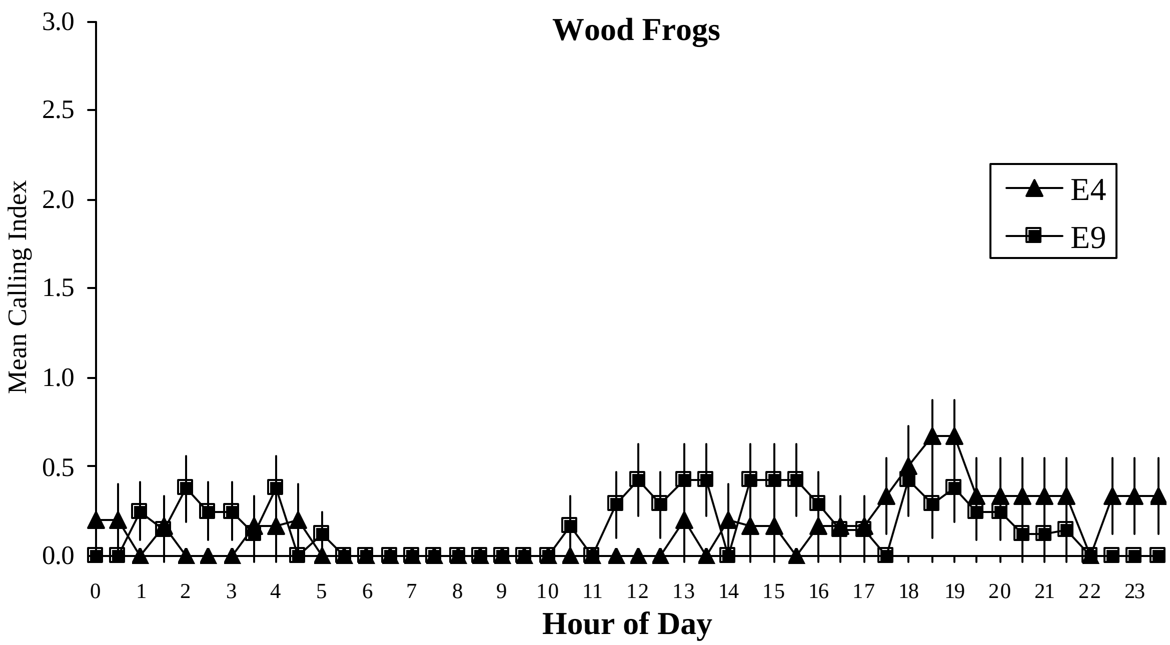

American Bullfrogs on Upper Pamet River
Mean Calling Index
3
2.5
2
1.5
1
0.5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Hours After Sunset


American Bullfrogs on Upper Pamet River
Mean Calling Index
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Hour of Day


Fowler's Toads at Motel Bog
Mean Calling Index
3
2.5
2
1.5
1
0.5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Hours After Sunset


Fowler's Toads at Motel Bog
Mean Calling Activity
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Hour of Day

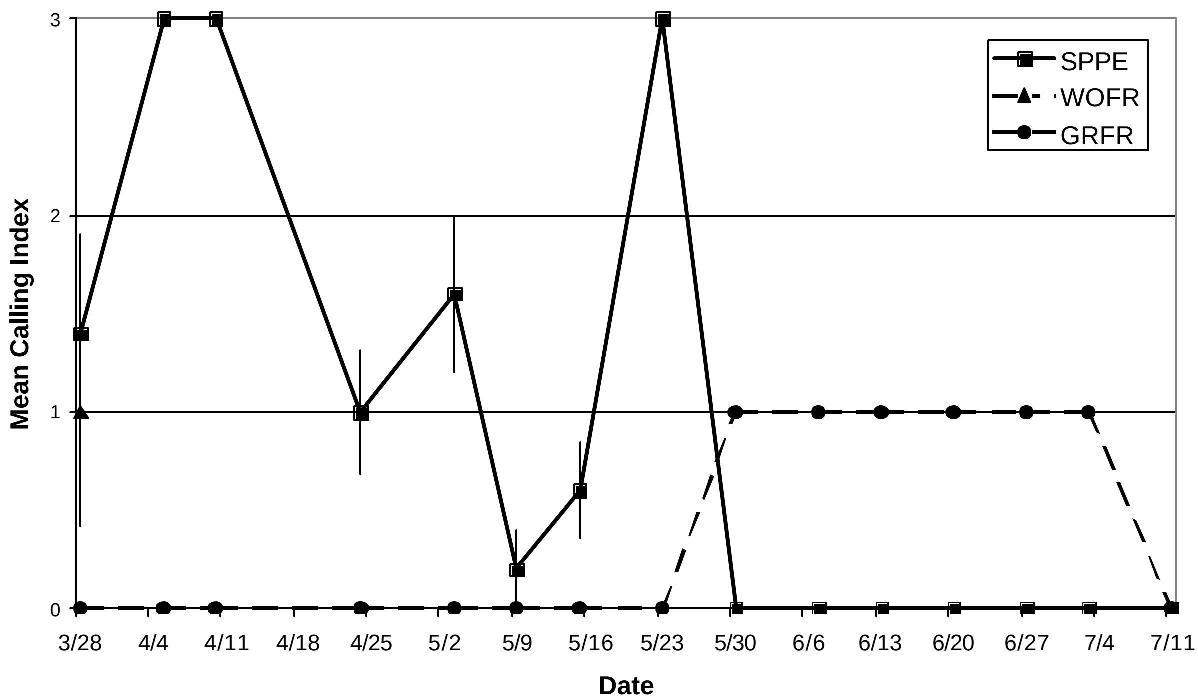

Wood Frogs
E4
E9
Mean Calling Index
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Hours After Sunset


Wood Frogs
E4
E9
Mean Calling Index
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Hour of Day

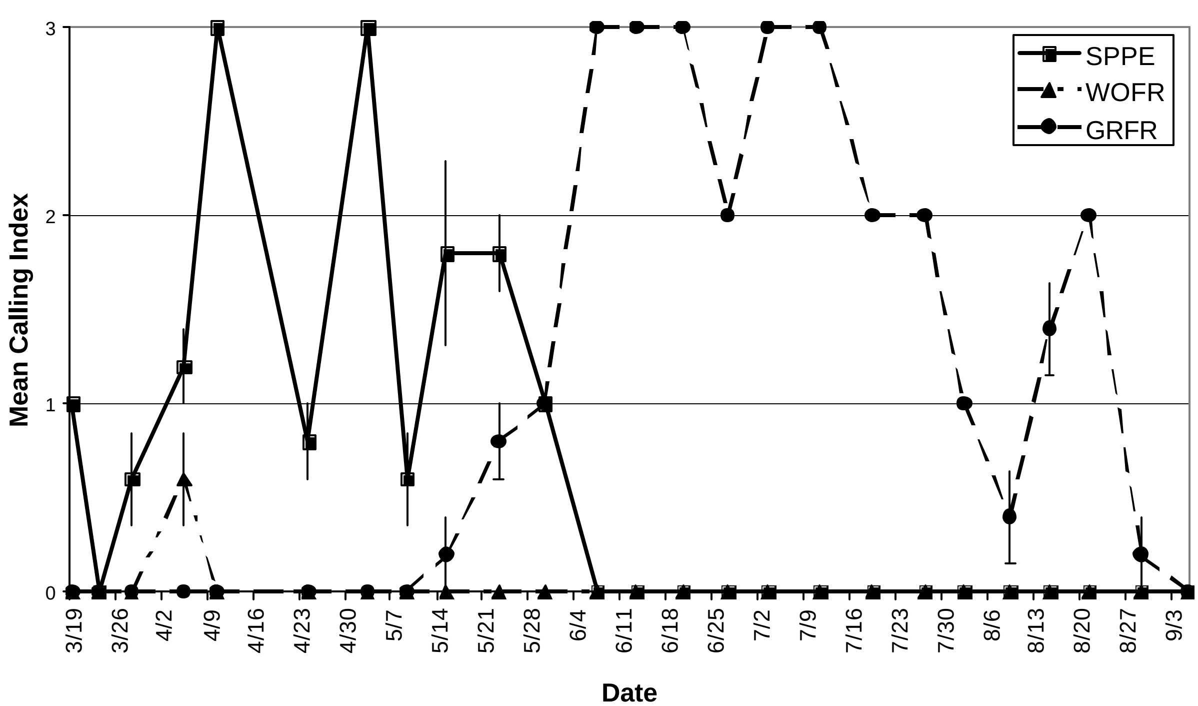
Appendix V. Seasonal variation in call chronology of anurans at all ponds monitored at Cape Cod NS during the 2001 field season.

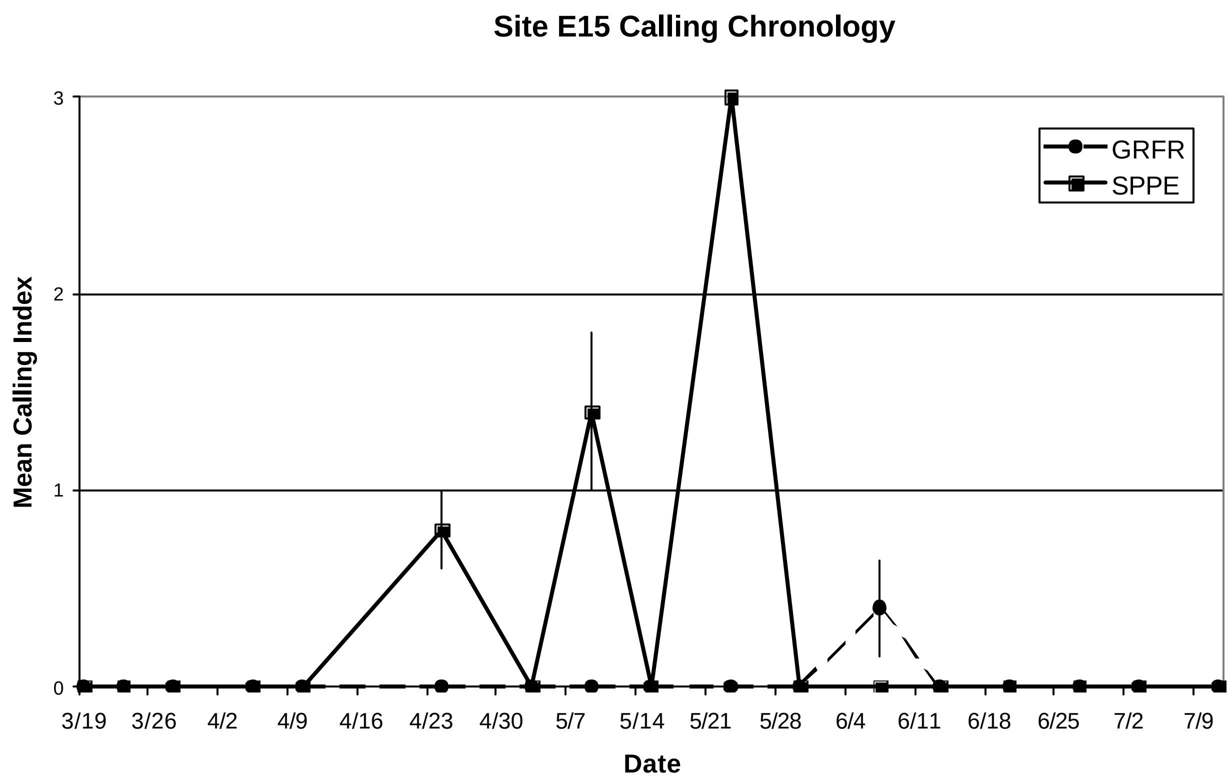
Species Codes: BUFR=American bullfrog, FOTO=Fowler's Toad, GRFR=green frog, PIFR=pickerel frog, SPPE=spring peeper, WOFR=wood frog.

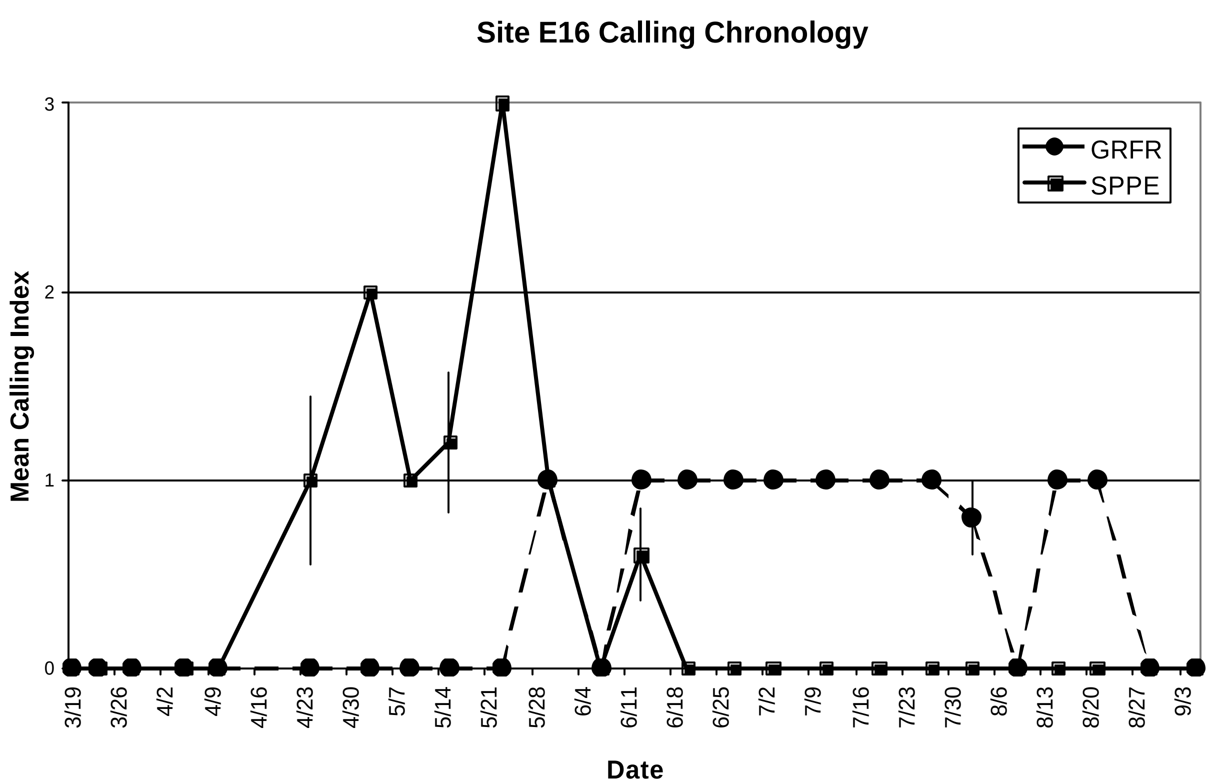
Site E4 Calling Chronology

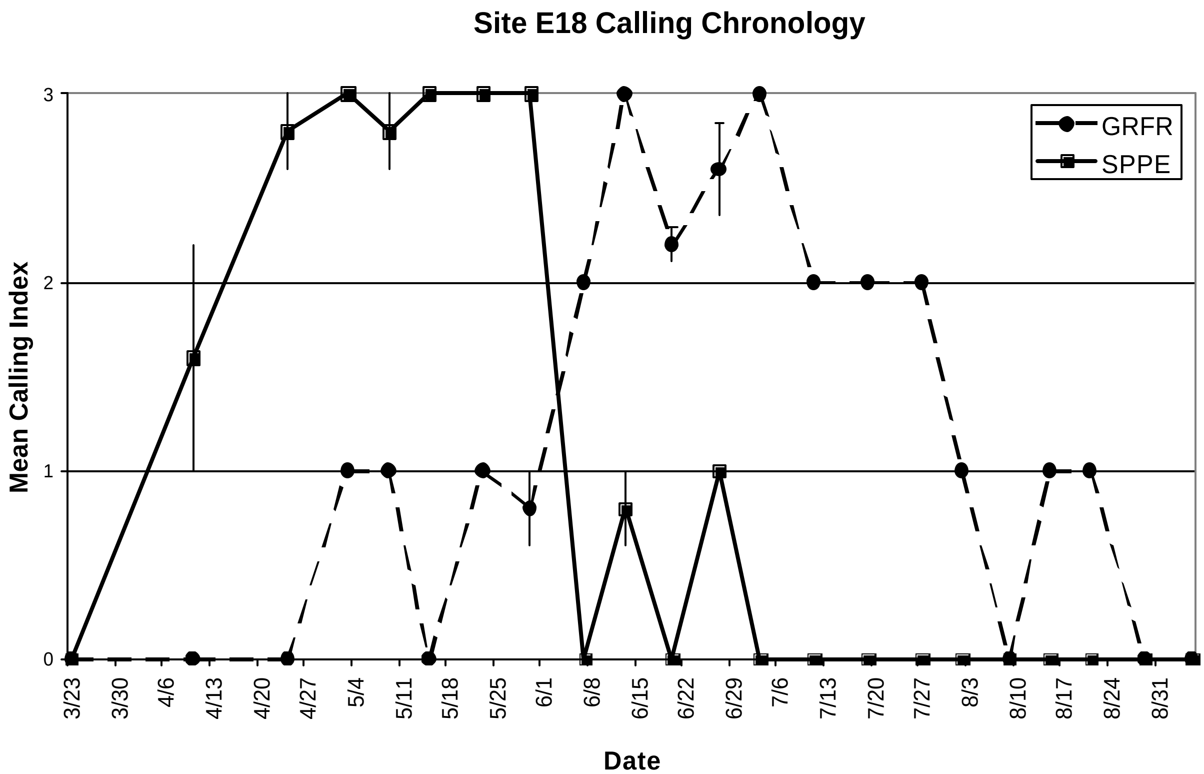


Site E9 Calling Chronology




Site E15 Calling Chronology
GRFR
SPPE
Mean Calling Index
0
1
2
3
3/19
3/26
4/2
4/9
4/16
4/23
4/30
5/7
5/14
5/21
5/28
6/4
6/11
6/18
6/25
7/2
7/9
Date


Site E16 Calling Chronology
GRFR
SPPE
Mean Calling Index
0
1
2
3
3/19
3/26
4/2
4/9
4/16
4/23
4/30
5/7
5/14
5/21
5/28
6/4
6/11
6/18
6/25
7/2
7/9
7/16
7/23
7/30
8/6
8/13
8/20
8/27
9/3
Date


Site E18 Calling Chronology
Mean Calling Index
Date
GRFR
SPPE
0
1
2
3
3/23
3/30
4/6
4/13
4/20
4/27
5/4
5/11
5/18
5/25
6/1
6/8
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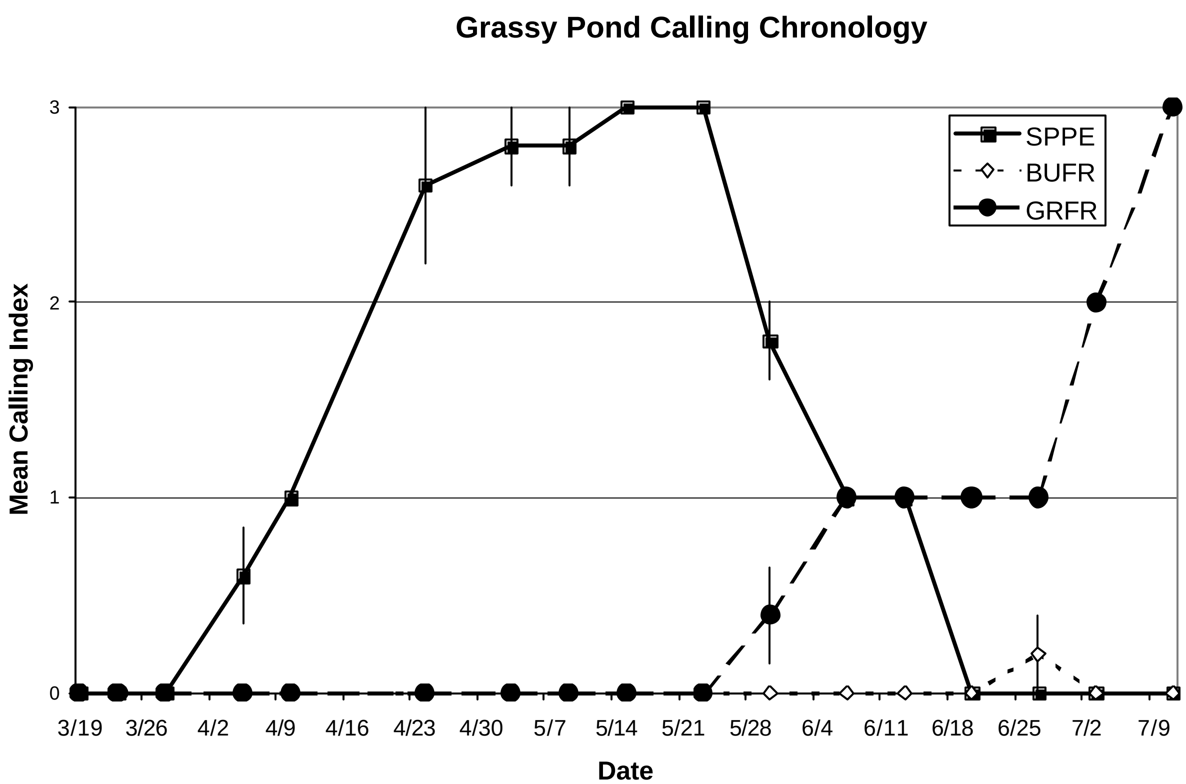
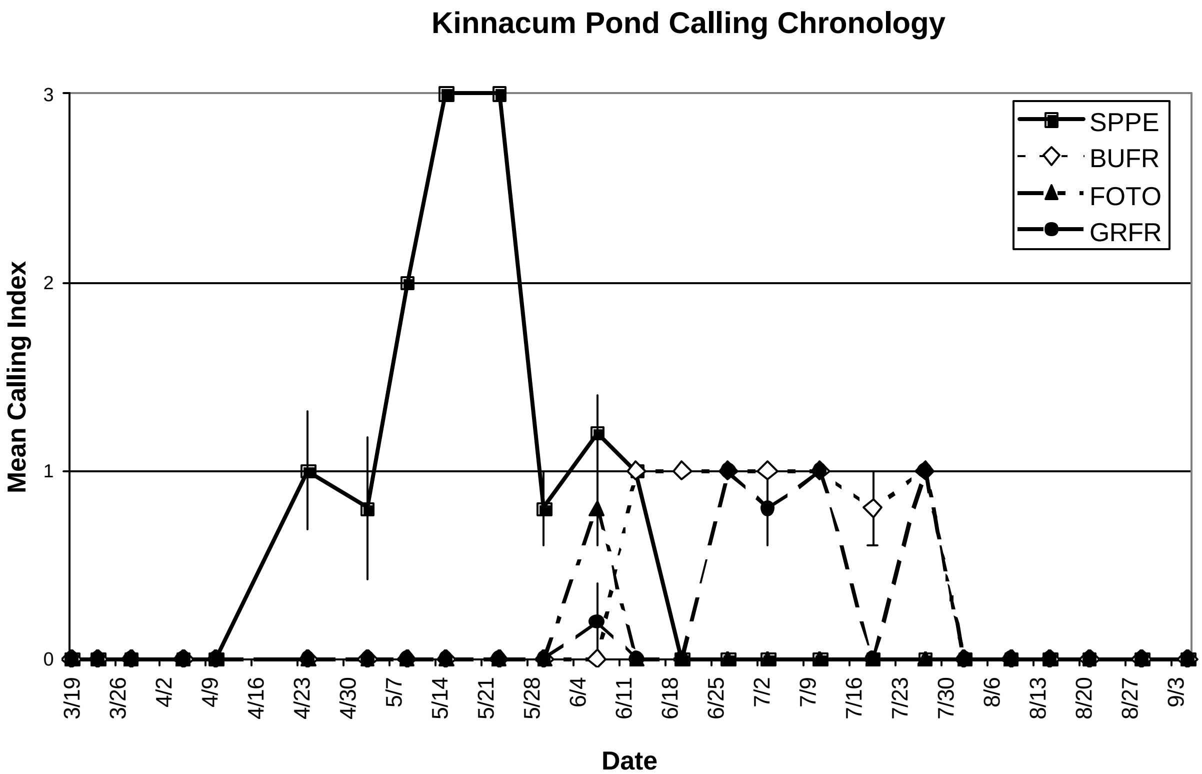
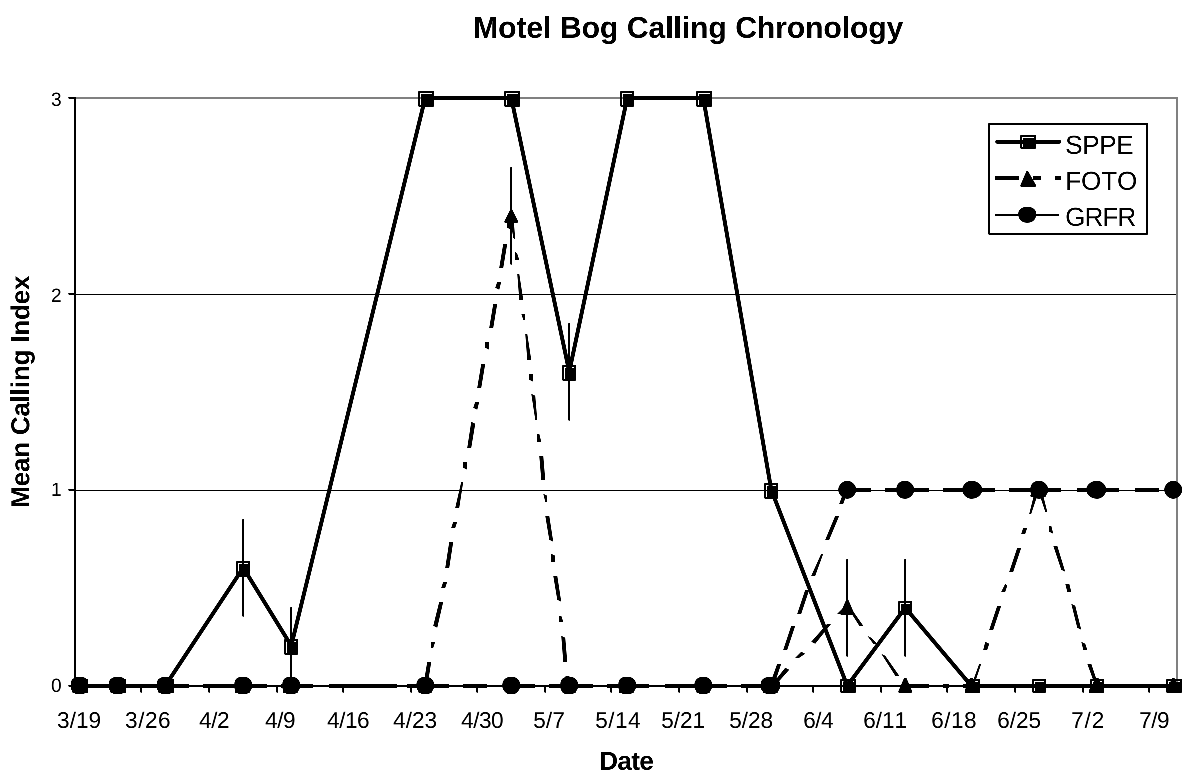
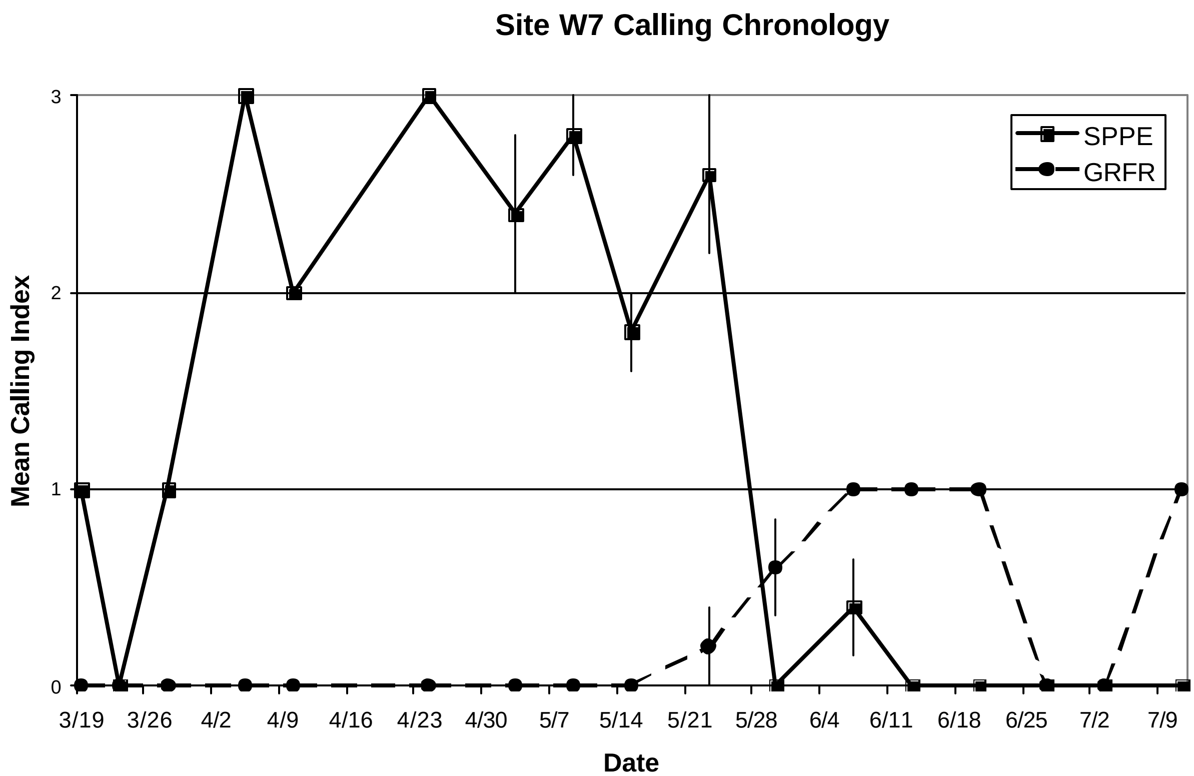
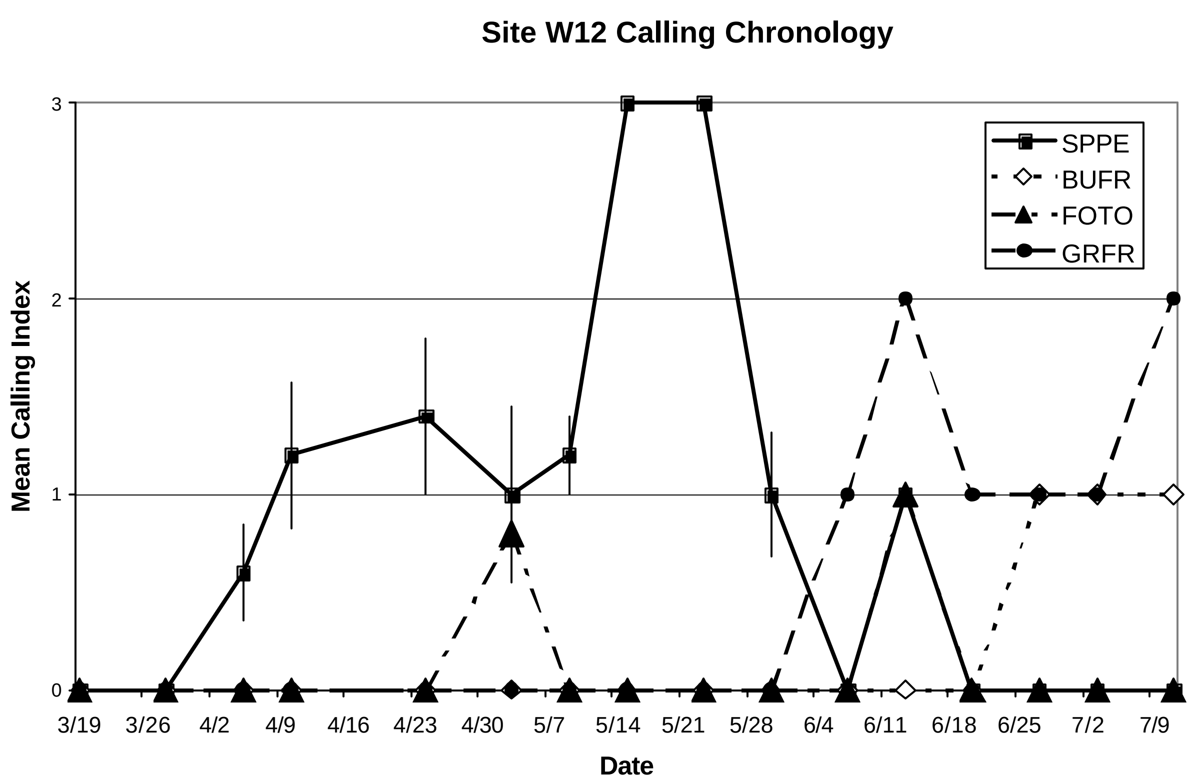
8/17
8/24
8/31

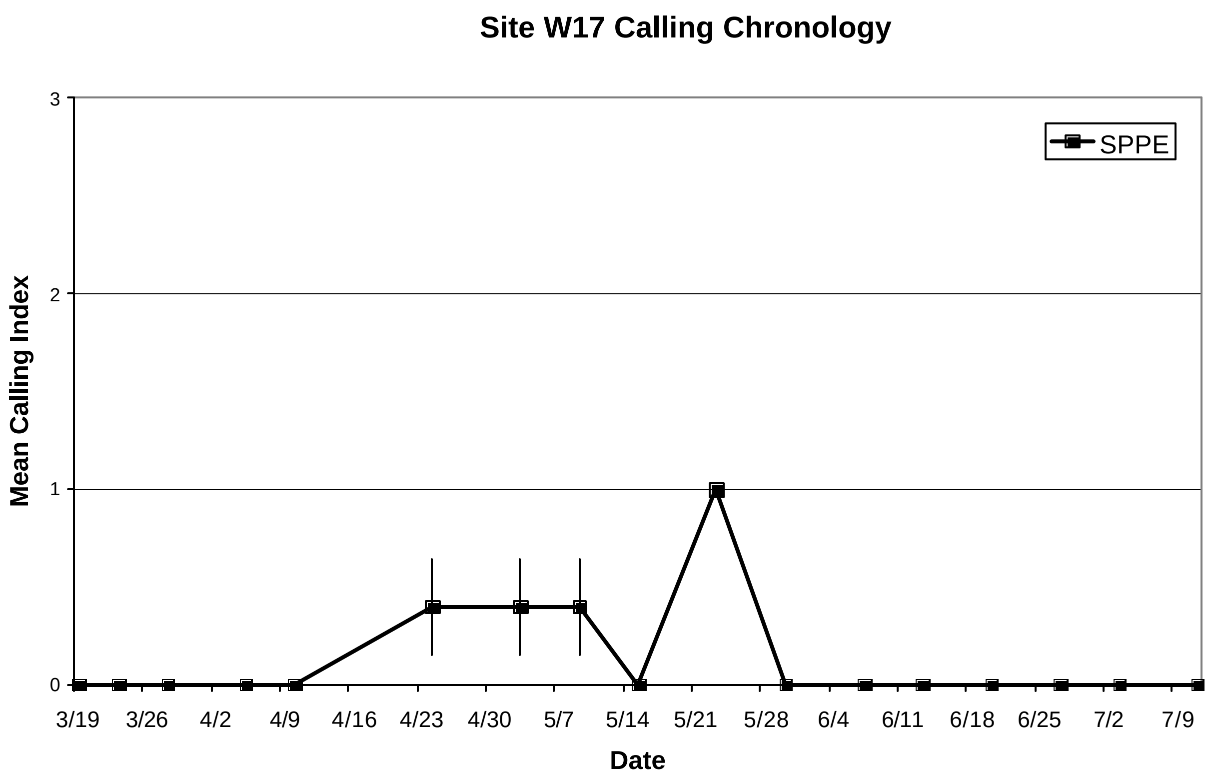

Grassy Pond Calling Chronology
Mean Calling Index
Date
SPPE
BUFR
GRFR
0
1
2
3
3/19
3/26
4/2
4/9
4/16
4/23
4/30
5/7
5/14
5/21
5/28
6/4
6/11
6/18
6/25
7/2
7/9

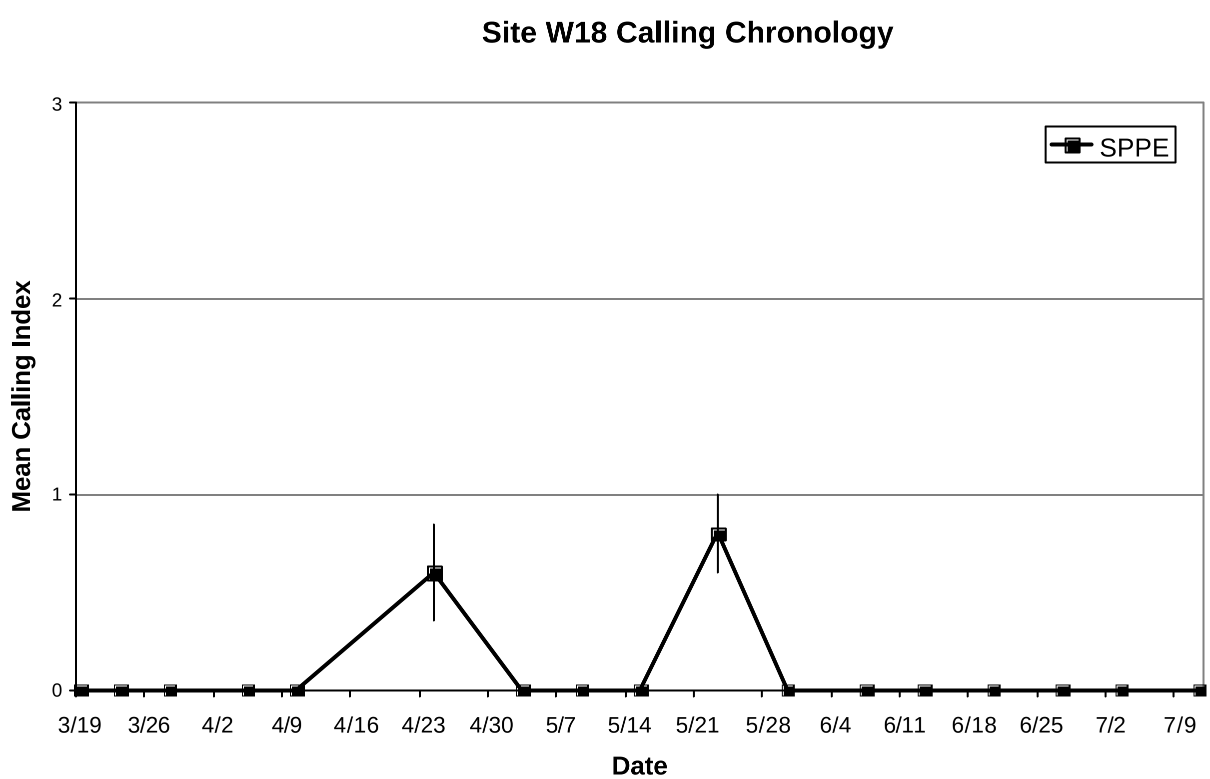

Kinnacum Pond Calling Chronology
Mean Calling Index
0
1
2
3
SPPE
BUFR
FOTO
GRFR
3/19
3/26
4/2
4/9
4/16
4/23
4/30
5/7
5/14
5/21
5/28
6/4
6/11
6/18
6/25
7/2
7/9
7/16
7/23
7/30
8/6
8/13
8/20
8/27
9/3
Date

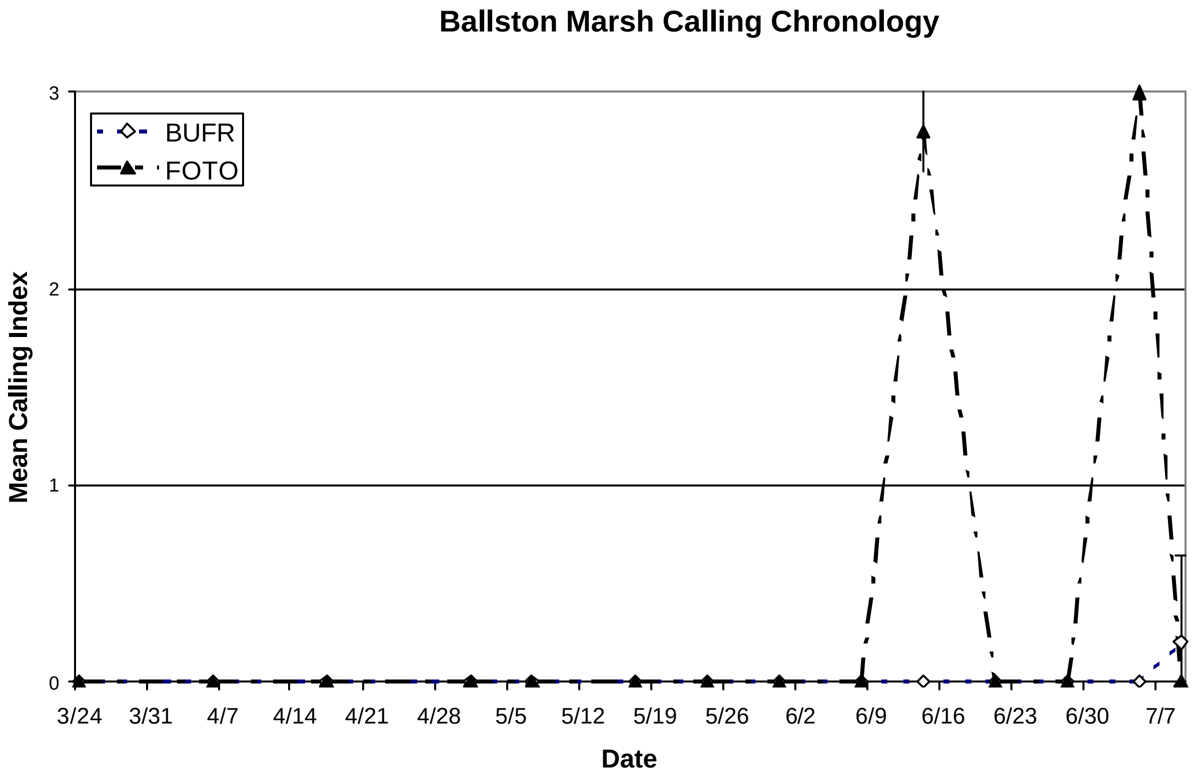

Motel Bog Calling Chronology
Mean Calling Index
0
1
2
3
SPPE
FOTO
GRFR
3/19
3/26
4/2
4/9
4/16
4/23
4/30
5/7
5/14
5/21
5/28
6/4
6/11
6/18
6/25
7/2
7/9
Date

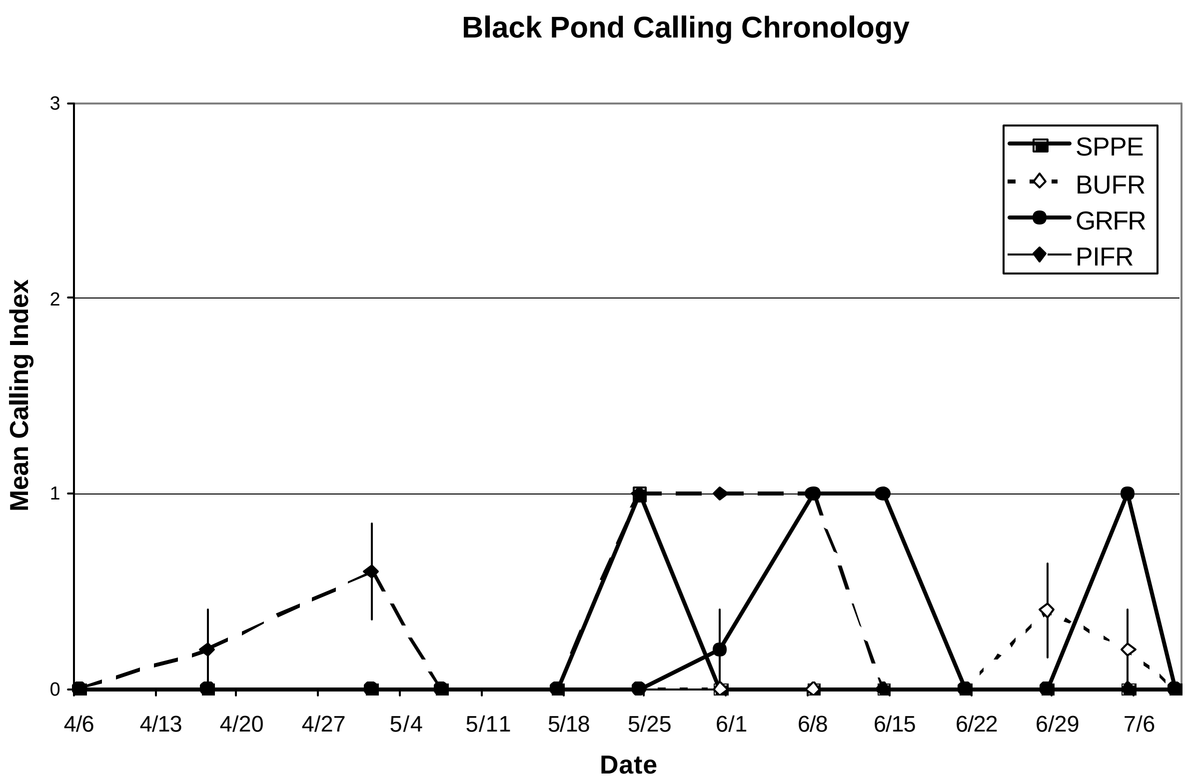

Site W7 Calling Chronology
Mean Calling Index
Date
SPPE
GRFR
0
1
2
3
3/19
3/26
4/2
4/9
4/16
4/23
4/30
5/7
5/14
5/21
5/28
6/4
6/11
6/18
6/25
7/2
7/9

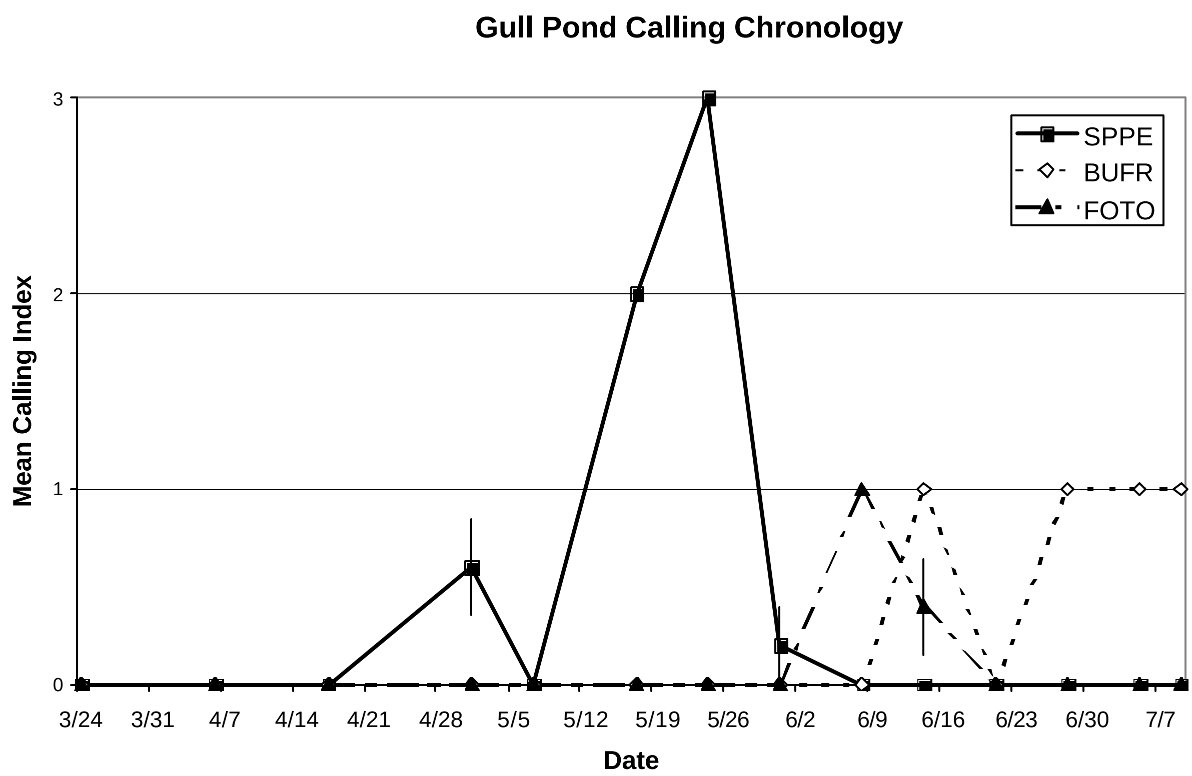

Site W12 Calling Chronology
Mean Calling Index
Date
SPPE
BUFR
FOTO
GRFR
0
1
2
3
3/19
3/26
4/2
4/9
4/16
4/23
4/30
5/7
5/14
5/21
5/28
6/4
6/11
6/18
6/25
7/2
7/9


Site W17 Calling Chronology
Mean Calling Index
0
1
2
3
SPPE
3/19
3/26
4/2
4/9
4/16
4/23
4/30
5/7
5/14
5/21
5/28
6/4
6/11
6/18
6/25
7/2
7/9
Date

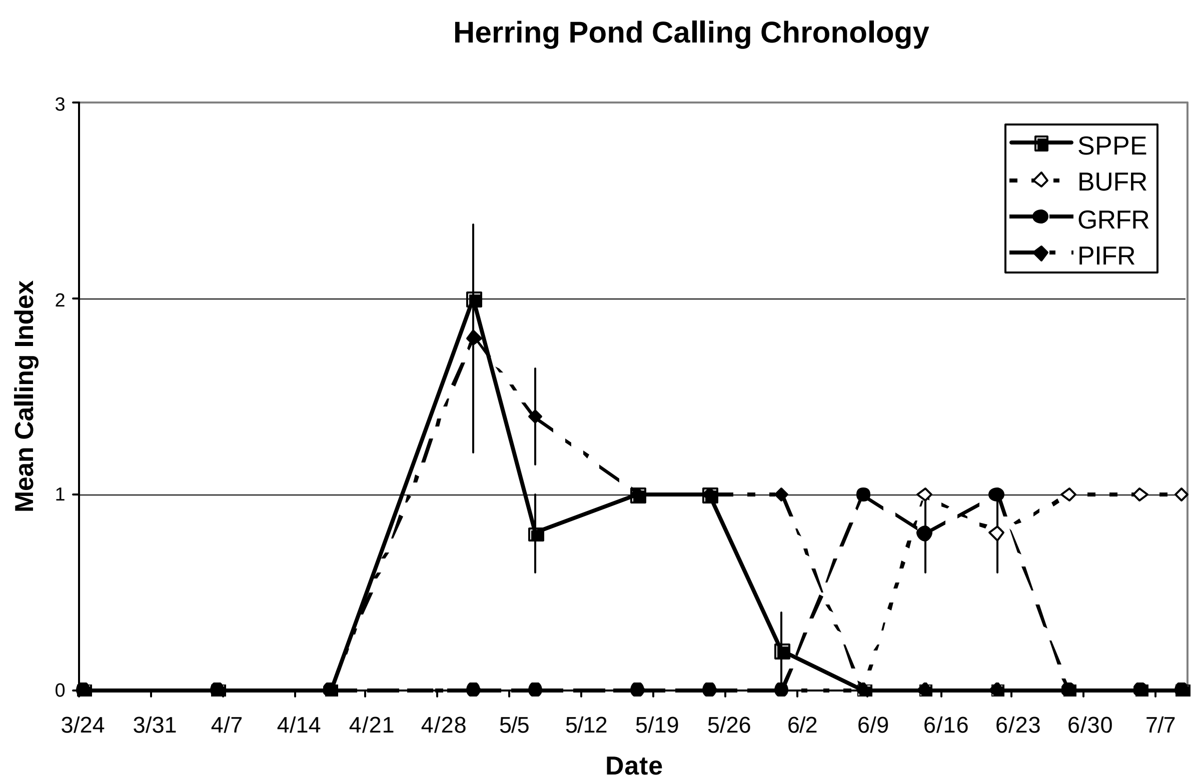

Site W18 Calling Chronology
Mean Calling Index
0
1
2
3
SPPE
3/19
3/26
4/2
4/9
4/16
4/23
4/30
5/7
5/14
5/21
5/28
6/4
6/11
6/18
6/25
7/2
7/9
Date


Ballston Marsh Calling Chronology
BUFR
FOTO
Mean Calling Index
0
1
2
3
3/24
3/31
4/7
4/14
4/21
4/28
5/5
5/12
5/19
5/26
6/2
6/9
6/16
6/23
6/30
7/7
Date


Black Pond Calling Chronology
SPPE
BUFR
GRFR
PIFR
Mean Calling Index
0
1
2
3
4/6
4/13
4/20
4/27
5/4
5/11
5/18
5/25
6/1
6/8
6/15
6/22
6/29
7/6
Date

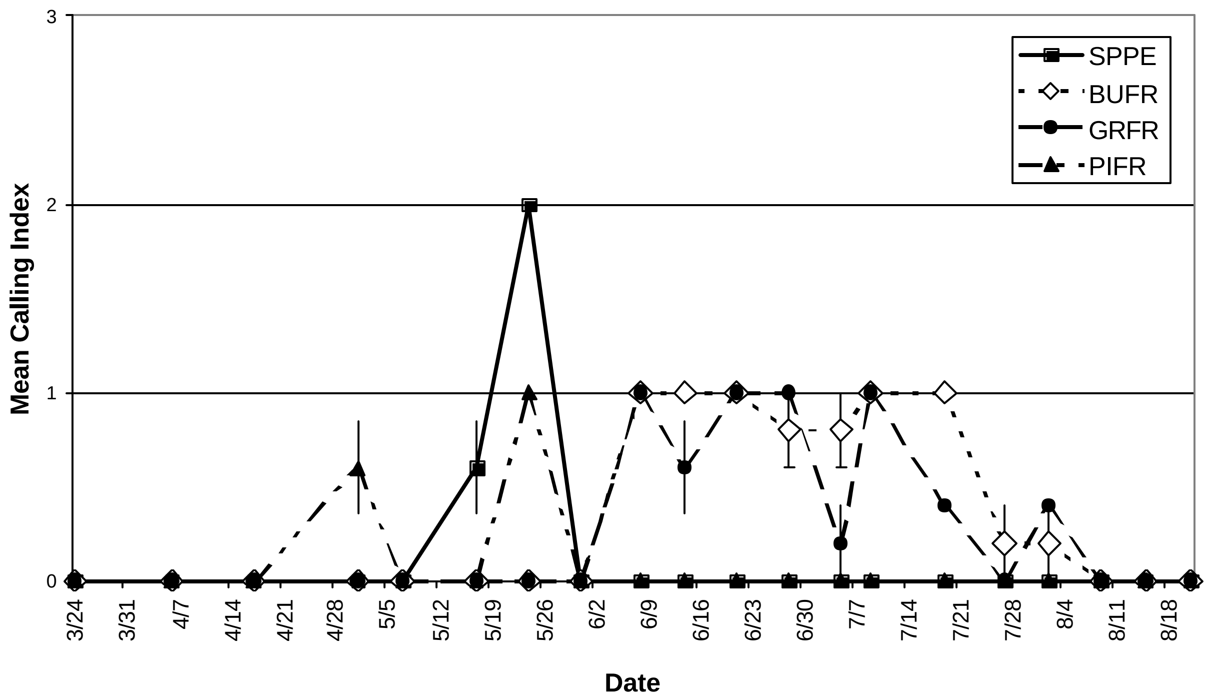


Gull Pond Calling Chronology
Mean Calling Index
0
1
2
3
SPPE
BUFR
FOTO
3/24
3/31
4/7
4/14
4/21
4/28
5/5
5/12
5/19
5/26
6/2
6/9
6/16
6/23
6/30
7/7
Date

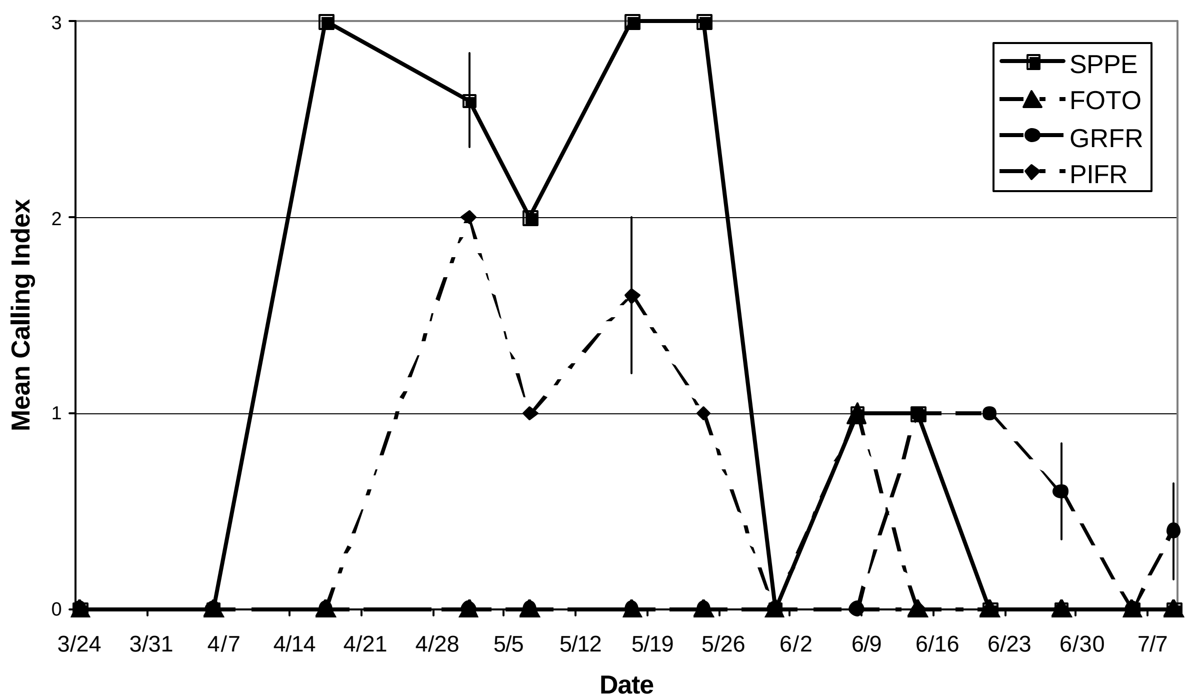


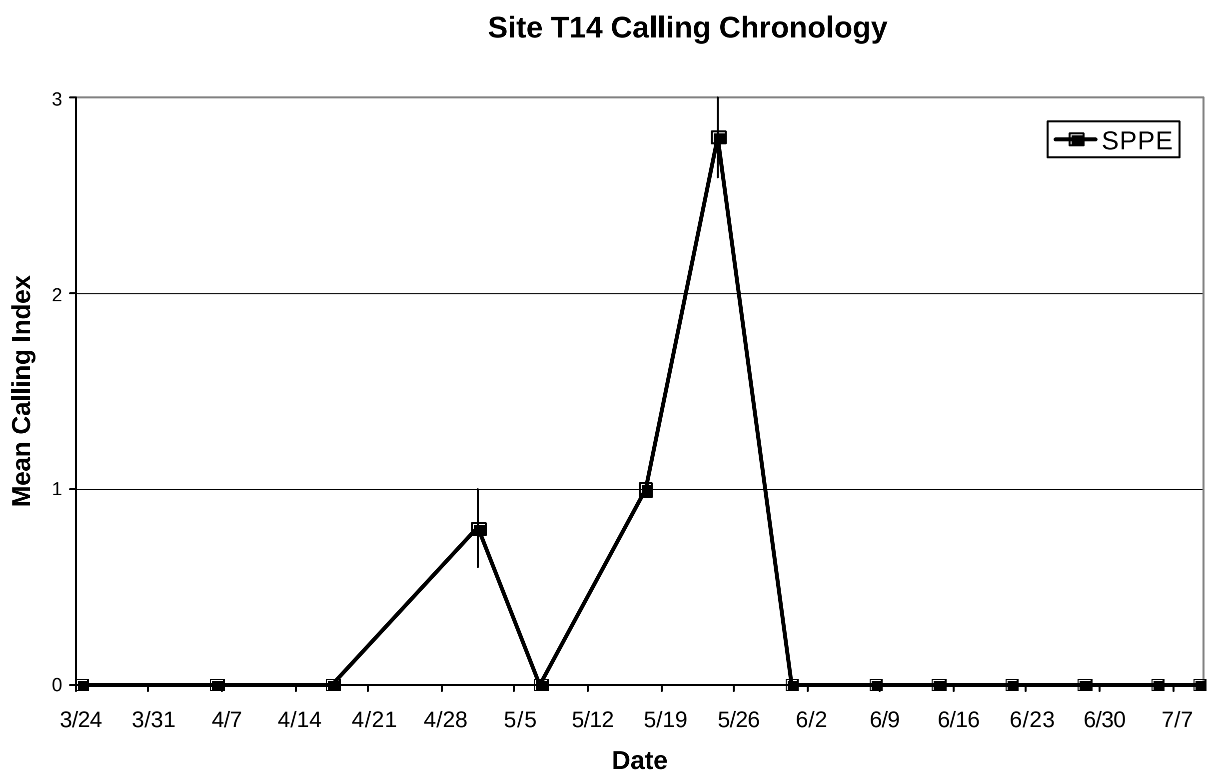
Herring Pond Calling Chronology
Mean Calling Index
0
1
2
3
SPPE
BUFR
GRFR
PIFR
3/24
3/31
4/7
4/14
4/21
4/28
5/5
5/12
5/19
5/26
6/2
6/9
6/16
6/23
6/30
7/7
Date

Pamet Bog Calling Chronology

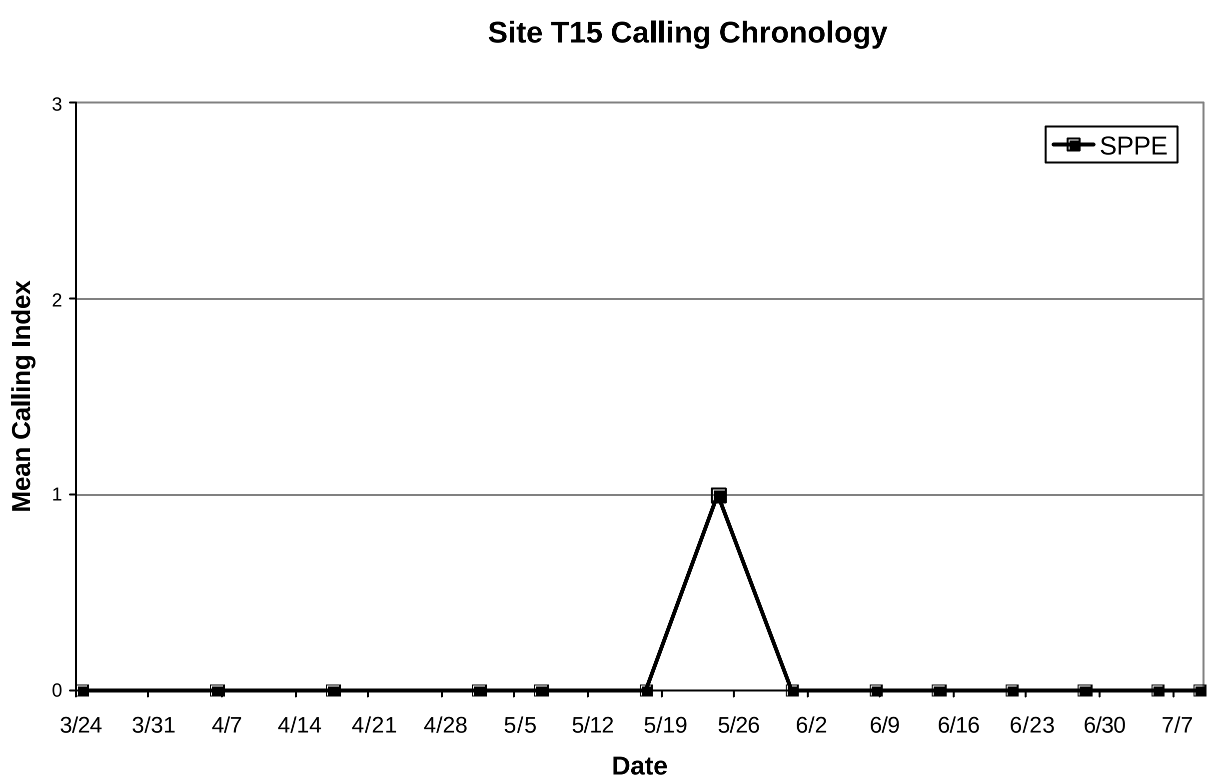


Snow Pond Calling Chronology



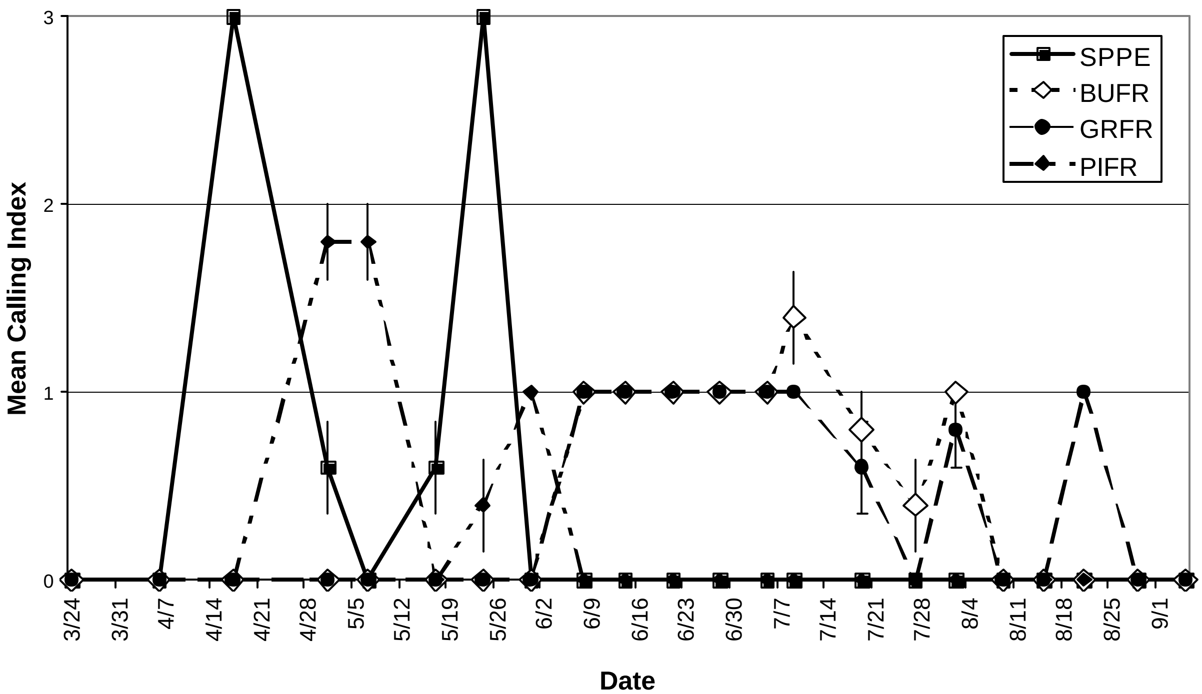


Site T14 Calling Chronology
SPPE
Mean Calling Index
0
1
2
3
3/24
3/31
4/7
4/14
4/21
4/28
5/5
5/12
5/19
5/26
6/2
6/9
6/16
6/23
6/30
7/7
Date

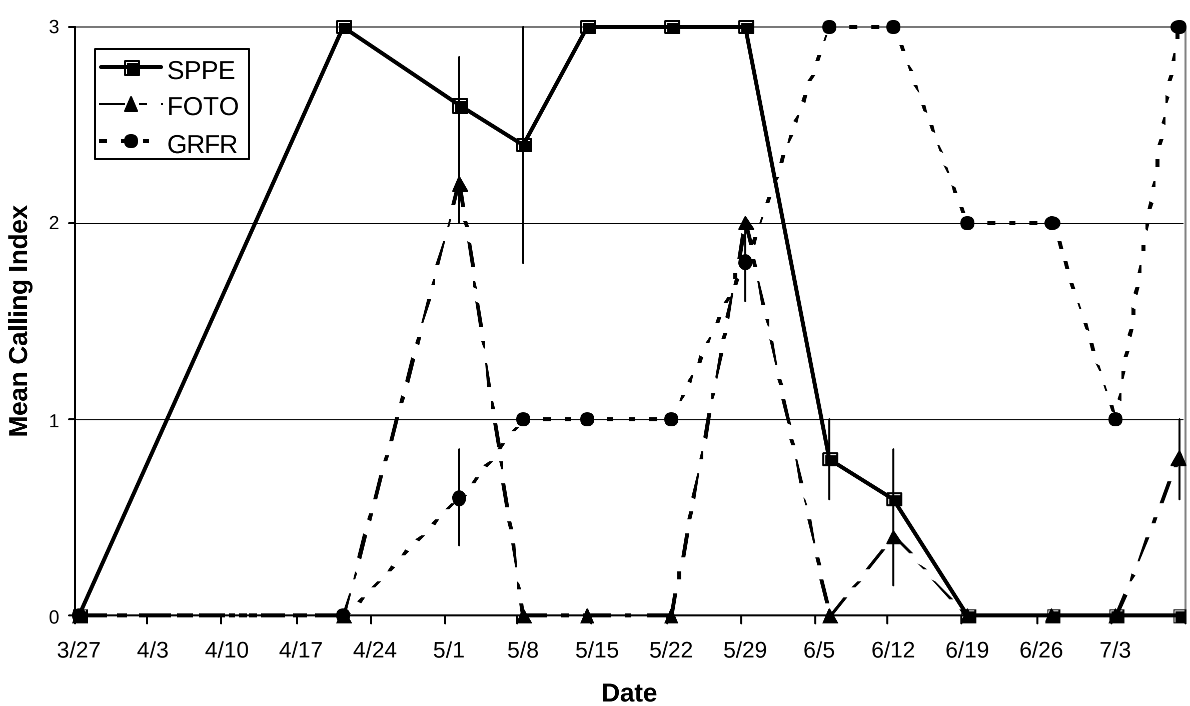


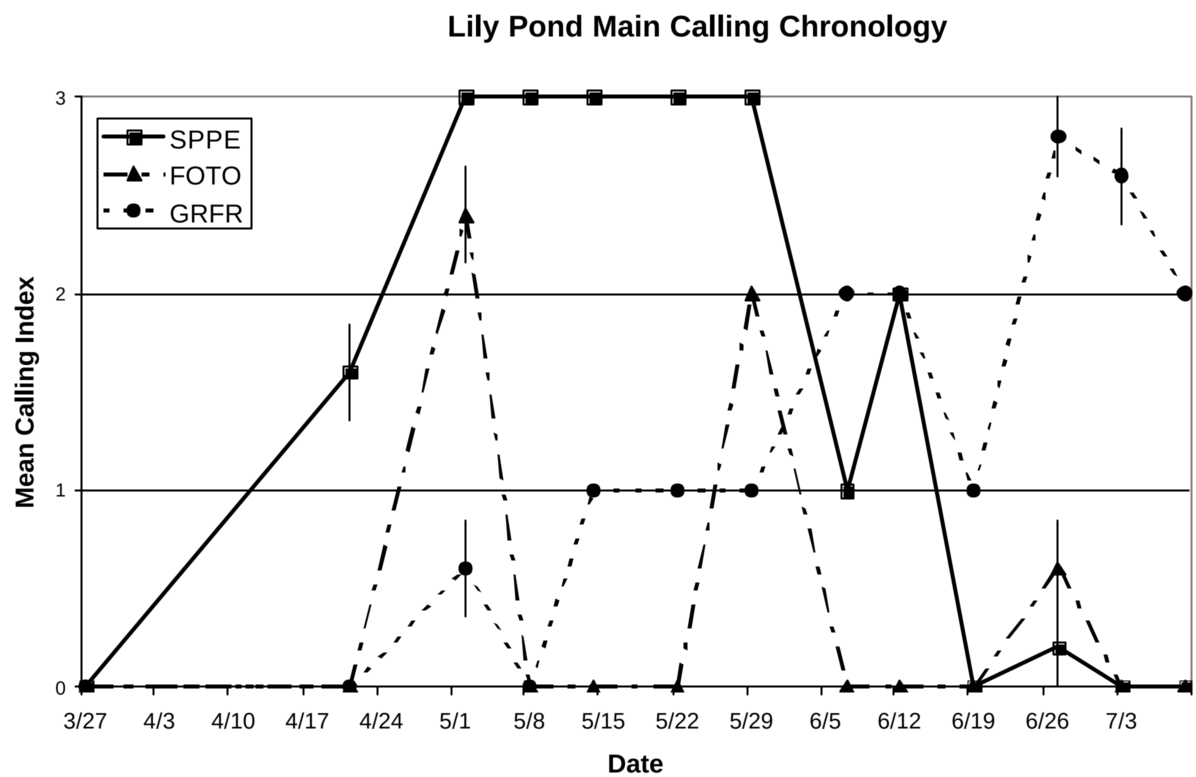
Site T15 Calling Chronology
SPPE
Mean Calling Index
0
1
2
3
3/24
3/31
4/7
4/14
4/21
4/28
5/5
5/12
5/19
5/26
6/2
6/9
6/16
6/23
6/30
7/7
Date

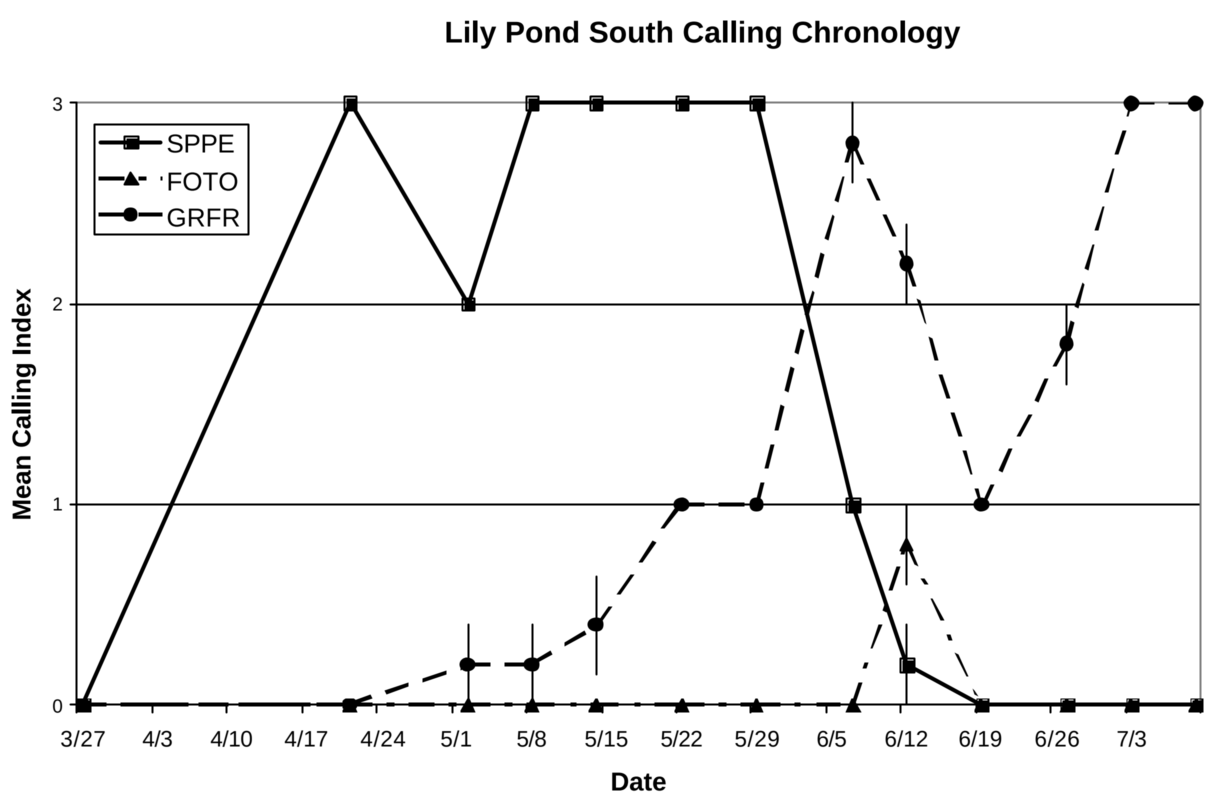
Upper Pamet Calling Chronology

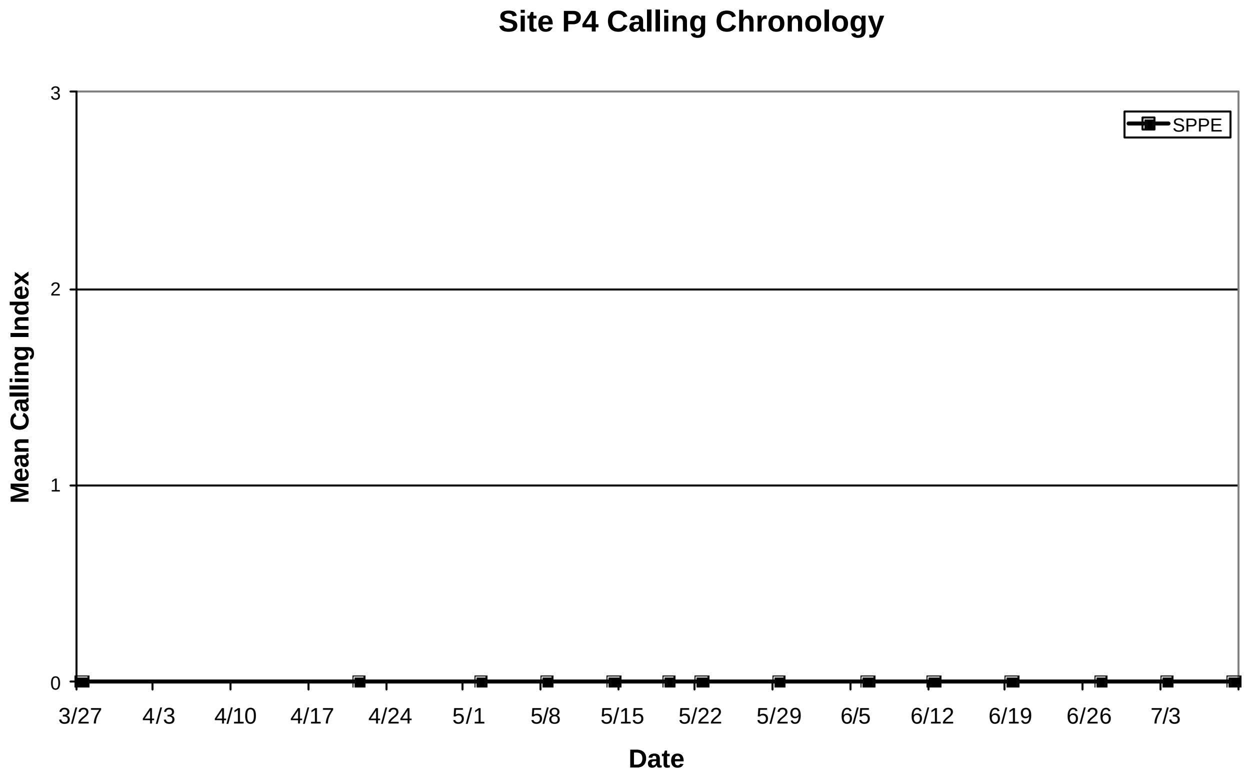


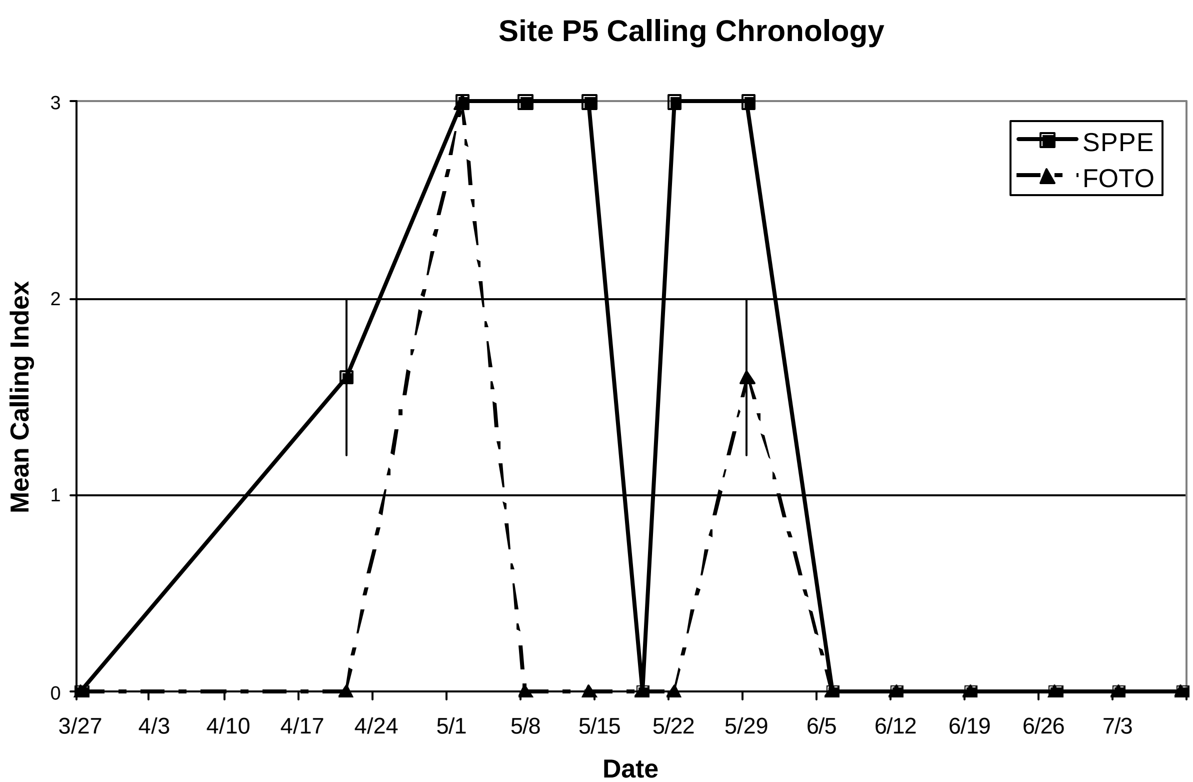
Great Pond Calling Chronology

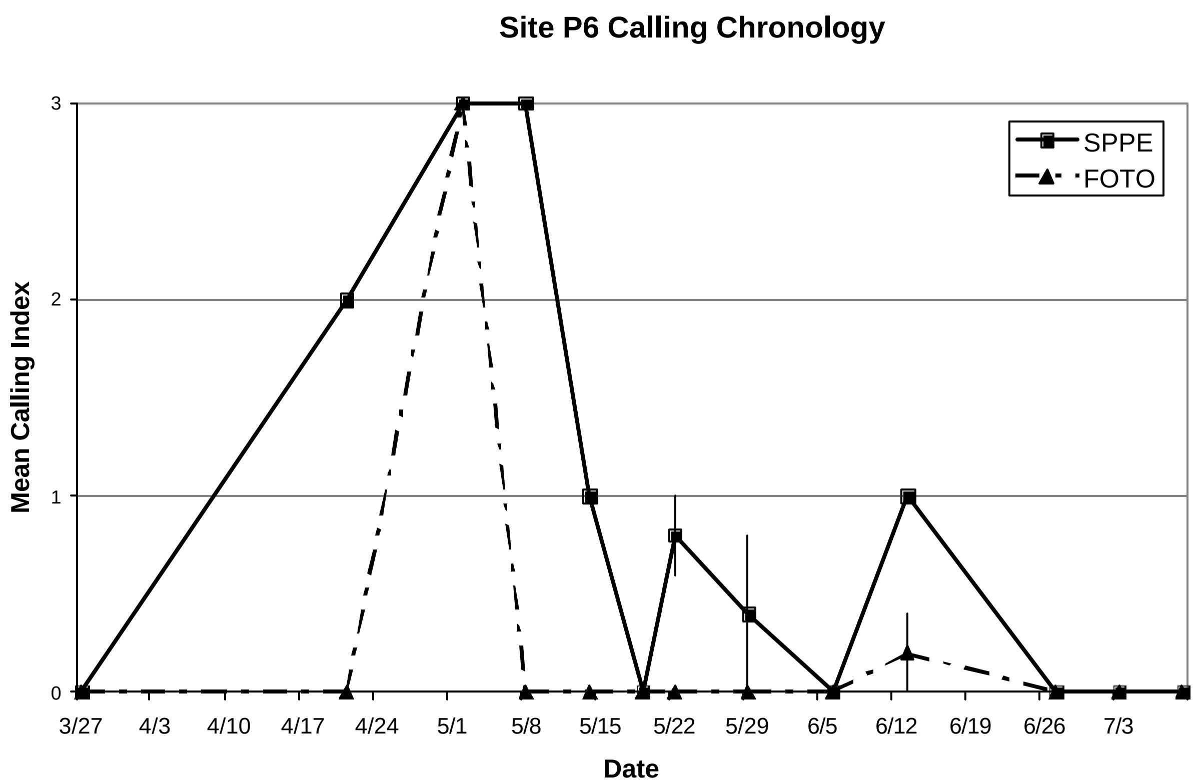


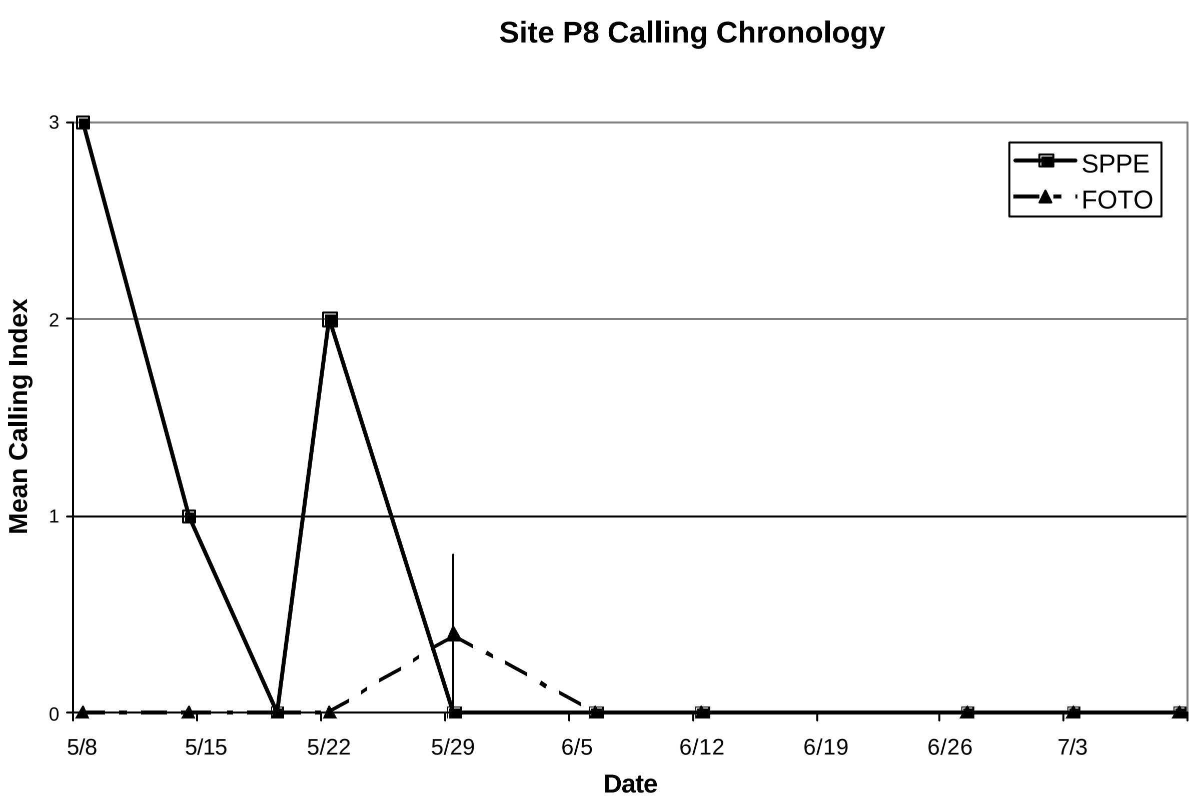

Lily Pond Main Calling Chronology
SPPE
FOTO
GRFR
Mean Calling Index
0
1
2
3
3/27
4/3
4/10
4/17
4/24
5/1
5/8
5/15
5/22
5/29
6/5
6/12
6/19
6/26
7/3
Date

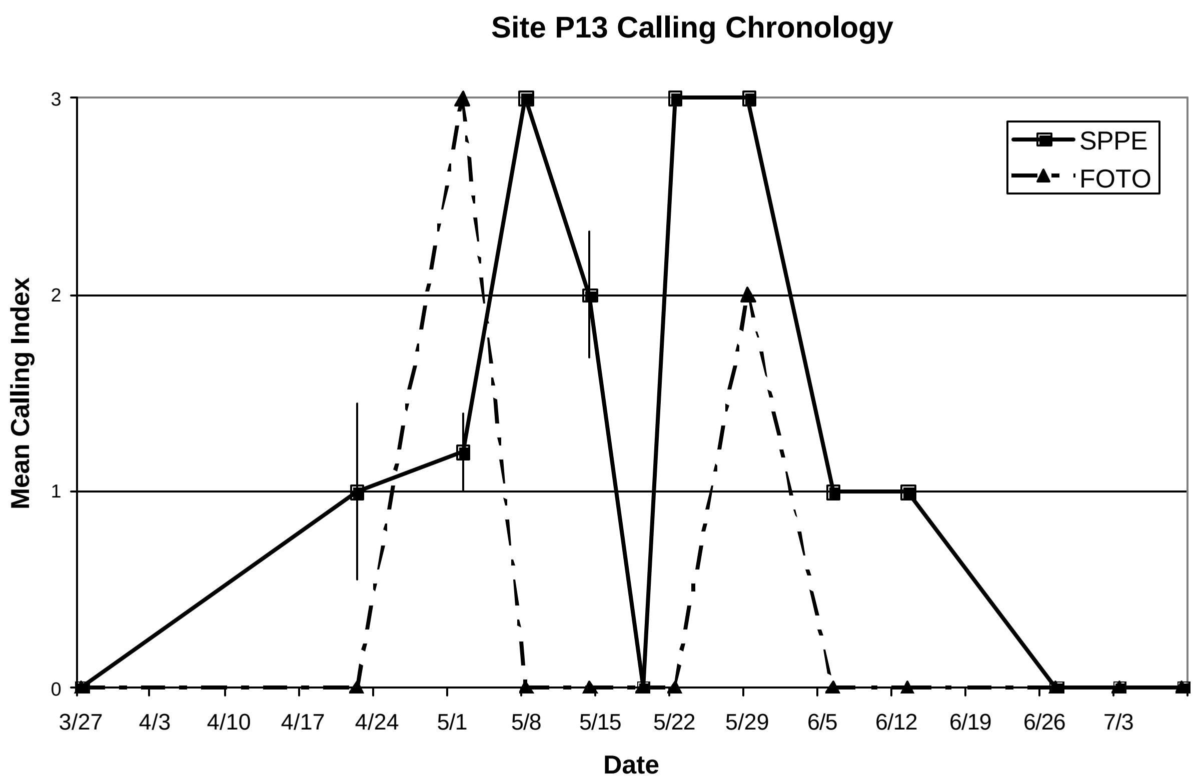

Lily Pond South Calling Chronology
SPPE
FOTO
GRFR
Mean Calling Index
0
1
2
3
3/27
4/3
4/10
4/17
4/24
5/1
5/8
5/15
5/22
5/29
6/5
6/12
6/19
6/26
7/3
Date

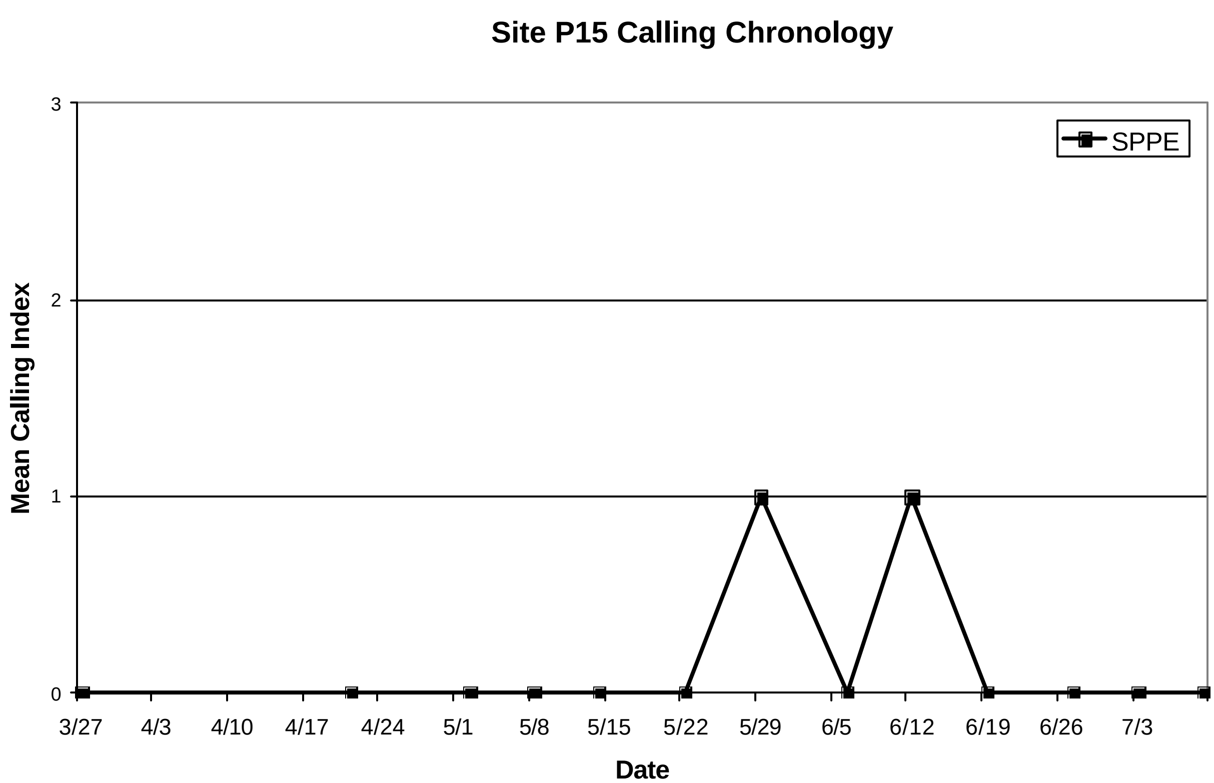

Site P4 Calling Chronology
SPPE
Mean Calling Index
0
1
2
3
3/27
4/3
4/10
4/17
4/24
5/1
5/8
5/15
5/22
5/29
6/5
6/12
6/19
6/26
7/3
Date

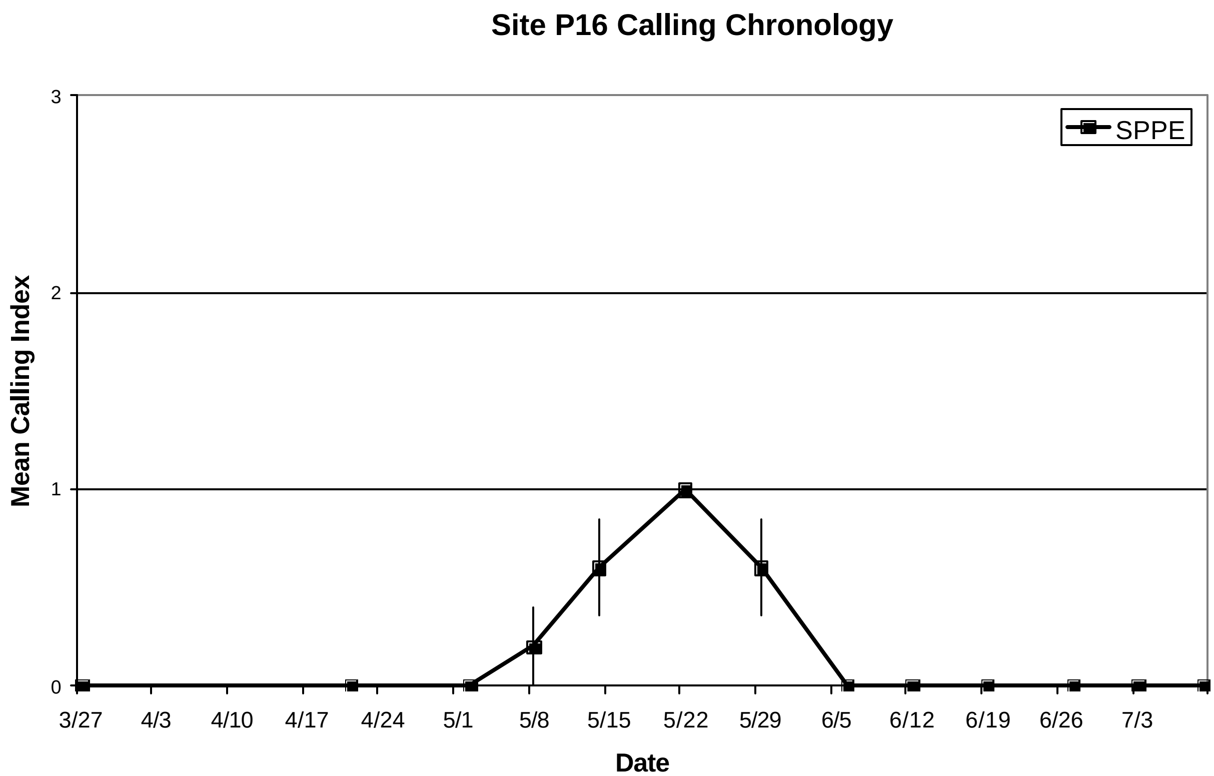

Site P5 Calling Chronology
SPPE
FOTO
Mean Calling Index
0
1
2
3
3/27
4/3
4/10
4/17
4/24
5/1
5/8
5/15
5/22
5/29
6/5
6/12
6/19
6/26
7/3
Date


Site P6 Calling Chronology
Mean Calling Index
3
2
1
0
SPPE
FOTO
3/27
4/3
4/10
4/17
4/24
5/1
5/8
5/15
5/22
5/29
6/5
6/12
6/19
6/26
7/3
Date


Site P8 Calling Chronology
Mean Calling Index
3
2
1
0
SPPE
FOTO
5/8
5/15
5/22
5/29
6/5
6/12
6/19
6/26
7/3
Date


Site P13 Calling Chronology
Mean Calling Index
3
2
1
0
Date
3/27
4/3
4/10
4/17
4/24
5/1
5/8
5/15
5/22
5/29
6/5
6/12
6/19
6/26
7/3
SPPE
FOTO


Site P15 Calling Chronology
Mean Calling Index
3
2
1
0
Date
3/27
4/3
4/10
4/17
4/24
5/1
5/8
5/15
5/22
5/29
6/5
6/12
6/19
6/26
7/3
SPPE


Site P16 Calling Chronology
Mean Calling Index
0
1
2
3
SPPE
3/27
4/3
4/10
4/17
4/24
5/1
5/8
5/15
5/22
5/29
6/5
6/12
6/19
6/26
7/3
Date

Appendix VI. Coordinates of Fowler's toads (FOTO) and eastern spadefoot toads (SPTO) found on roads during fieldwork at Cape Cod NS during 2001 field season.

Lat. (UTM)	Long. (UTM)	# Indivs.	SPP	Lat. (UTM)	Long. (UTM)	# Indivs.	SPP
0418958	4641413	NA	FOTO	0400066	4658509	NA	FOTO
0416901	4643453	NA	FOTO	0400005	4658690	NA	FOTO
0416916	4643087	NA	FOTO	0399777	4659050	NA	FOTO
0417431	4644996	NA	FOTO	0399576	4658969	NA	FOTO
0416120	4645357	NA	FOTO	0399984	4658747	NA	FOTO
0414887	4646816	NA	FOTO	0399934	4658831	NA	FOTO
0400801	4657799	NA	FOTO	0399860	4658869	NA	FOTO
4041061	4657857	NA	FOTO	0399860	4658869	NA	FOTO
0404001	4657770	NA	FOTO	0400018	4658515	NA	FOTO
0403711	4657774	NA	FOTO	0399915	4658828	NA	FOTO
0401978	4657279	NA	FOTO	0401178	4657884	NA	FOTO
0401526	4657135	NA	FOTO	0400256	4658421	NA	FOTO
0400447	4658582	NA	FOTO	0399857	4658332	NA	FOTO
0401399	4657374	NA	FOTO	0401405	4657852	NA	FOTO
0400640	4658475	NA	FOTO	0400610	4658421	NA	FOTO
0419915	4633593	NA	FOTO	0418917	4641758	NA	FOTO
0417809	4644867	NA	FOTO	0418900	4641722	NA	FOTO
0417505	4645268	NA	FOTO	0418108	4637057	NA	FOTO
0416990	4645139	NA	FOTO	0418873	4642027	NA	FOTO
0417274	464550	NA	FOTO	0418738	4642896	NA	FOTO
0417909	4644561	NA	FOTO	0418542	4643342	NA	FOTO
0418078	4644088	NA	FOTO	0418343	4643836	NA	FOTO
0418773	4644088	NA	FOTO	0417944	4644374	NA	FOTO
0418710	4643016	NA	FOTO	4017804	4644870	NA	FOTO

Appendix VI. *continued*.

Lat. (UTM)	Long. (UTM)	# Indivs.	SPP	Lat. (UTM)	Long. (UTM)	# Indivs.	SPP
0412152	4650978	1	SPTO	0401353	4657824	1	SPTO
0405111	4657558	1	SPTO	0401401	4657861	1	SPTO
0404978	4657641	3	SPTO	0401409	4657915	1	SPTO
0404788	4657701	1	SPTO	0401338	4657792	2	SPTO
0401352	4657535	1	SPTO	0401299	4657947	1	SPTO
0401338	4657624	1	SPTO	0401234	4658060	1	SPTO
0401234	4658001	3	SPTO	0401863	4657164	2	SPTO
0400748	4658402	1	SPTO	0402955	4657581	2	SPTO
0400354	4658601	3	SPTO	0411589	4654974	1	SPTO
0400237	4658552	2	SPTO	0404895	4657681	1	SPTO
0400180	4658541	1	SPTO	0404019	4657785	1	SPTO
0400058	4658483	1	SPTO	0401328	4657739	1	SPTO
0399844	4658124	1	SPTO	0401316	4657831	1	SPTO
0399426	4657712	1	SPTO	0401228	4658023	2	SPTO
0399390	4657606	1	SPTO	0401120	4658193	3	SPTO
0399307	4657359	1	SPTO	0410157	4654591	1	SPTO
0399178	4656455	3	SPTO	0404895	4657681	4	SPTO
0398957	4656177	1	SPTO	0404019	4657775	3	SPTO
0399312	4655598	2	SPTO	0401977	4657283	1	SPTO
0398984	4656242	1	SPTO	0401228	4658023	1	SPTO
0399082	4657322	2	SPTO	0400772	4658400	1	SPTO
0399690	4657994	1	SPTO	0400606	4658507	1	SPTO
0399916	4658835	2	SPTO	0401401	4657375	1	SPTO
0399786	4658884	1	SPTO	0404381	4657711	1	SPTO
0399465	4659020	1	SPTO	0402676	4637898	1	SPTO
0399300	4659132	1	SPTO	0411185	4652641	1	SPTO
0399663	4658930	1	SPTO	0405034	4657584	1	SPTO
0399780	4658886	2	SPTO	0411300	4651983	3	SPTO
0400258	4658560	1	SPTO	0411728	4651274	1	SPTO
0400686	4658452	1	SPTO	0411915	4651116	1	SPTO
0401108	4658205	1	SPTO	0411979	4651077	1	SPTO
0401180	4658141	1	SPTO	0412216	4650945	1	SPTO
0401234	4658060	1	SPTO	0411195	4652617	1	SPTO
0401299	4657947	1	SPTO	0418750	4640381	1	SPTO
0401016	4658263	1	SPTO	0408744	4655640	1	SPTO
0400772	4658400	2	SPTO	0418889	4636970	1	SPTO
0400486	4658563	1	SPTO	0418575	4636313	1	SPTO
0400288	4658573	2	SPTO	0399849	4658870	1	SPTO
0399963	4658311	2	SPTO	0399999	4658672	1	SPTO
0399806	4651803	1	SPTO	0400653	4658499	1	SPTO
0399435	4657810	1	SPTO	0401074	4658219	1	SPTO
0399292	4657382	1	SPTO	0401149	4658139	1	SPTO
0399098	4657318	5	SPTO	0401172	4658087	1	SPTO
0399879	4658236	2	SPTO	0400421	4658575	1	SPTO
0402820	4657446	1	SPTO	0400724	4658418	1	SPTO
0404128	4657727	1	SPTO				